Microbial mineralization processes influenced by water table changes and peat quality in an acidic fen

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CONTENTS

INTRODUCTION

Characteristics and classification of peatlands

Peatlands are a diverse group of wetland ecosystems that are broadly characterized by an imbalance between production of plant biomass and slow degradation processes [Moore & Bellamy 1974]. This imbalance is caused by waterlogging, resulting in anoxic soil conditions, which leads to the formation of soil organic matter, called peat. Peatlands are areas where peat layers are thicker than 30 cm and have a dry weight (dry wt) organic matter content greater than 30% [Glaser & Janssens 1986]. Several kinds of peatlands have been distinguished based on the characteristics of their vegetation, geomorphology, hydrology, chemistry, stratigraphy, and peat, resulting in extensive classification systems [Mitsch & Gosselink 2000, Succow & Joosten 2001].

The two main peatland types, which differ in their hydrology and mineral status, are precipitation-fed (ombrotrophic) bogs and precipitation as well as groundwater-fed (minerotrophic) fens. The surface of ombrogenous peat is above the surrounding land and peat layers are up to 20 m deep [Whitmore 1984, Whitten et al. 1987]. The peat and drainage water is very low in nutrients, as no nutrients enter the system from the mineral soil or ground water. Thus, the vegetation exists solely on nutrients from the living biomass, peat or from rainwater. Peat-forming mosses like *Sphagnum* spp. are typically found in bogs and exclude protons (H^+), thereby creating an acidic environment (pH < 5) [Wheeler & Proctor 2000]. In contrast, minerotrophous peat is formed in topographic depressions, and plants receive nutrients from the mineral subsoil and groundwater, in addition to plant residues and rainwater. Thus, the nutrient levels in minerotrophous peatlands range from oligotrophic to eutrophic but are typically mesotrophic. The soil pH (usually 4 to 9) can be higher than that of ombrogenous peat and is more favorable for soil microorganisms, which are involved in the mineralization of soil organic matter. Many plant species are able to reach the mineral silt and clay below the peat and are thus not entirely dependent on rainwater for nutrients. Nonetheless, mostly low productive nutrient-limited vegetation like *Cyperaceae* and *Bryophyta* are characteristic for fens [Hajek et al. 2006]. Peat development in fens is slower than observed for bogs, and great depths of peat are usually not formed [Whitten et al. 1987].

Because of the challenging ecological conditions of peatland ecosystems, they are home to many rare and specialized organisms. Peatland plants, in particular, must deal with limited oxygen availability, acidic conditions, and the lack of essential nutrients and have developed a variety of adaptations that allow them to survive under these conditions.

Aerenchyms of several vascular plants are used to transport gases like oxygen into their root system to tolerate anoxic conditions. The perhaps most spectacular and best-known adaptation to nutrient limitation are carnivorous plants, i.e. sundews (*Drosera* spp.), that derive some or most of their nutrients by trapping and consuming arthropods.

Sink and source function of peatlands

Peatlands have functioned as sinks for carbon (C) since the end of the last glacial period (c.a. 11,000 years ago), due to the rate of plant biomass production generally exceeding the rate of organic matter decomposition over the millennia [Clymo 1984]. This illustrates the potential for large C release if peatlands were destabilized by global climate change. Boreal and subarctic regions contain the largest areas of peatlands, although some are found in more temperate and even tropical parts of the world [Gorham 1991, Sorensen 1993]. Peatlands are important C sinks even though their net primary production (NPP) is low relative to other ecosystems [Blodau 2002]. This is due to the fact that below ground NPP is an enormous contributor to overall NPP, as 30-50% of the production by vascular plants occurs within the soil [Blodau 2002]. Production of plant biomass is the primary source for the formation of peat organic matter and there is far more carbon in the below ground peat (~98.5%) than in the surface vegetation [Gorham 1991]. Variations in the characteristics (e.g., productivity, litter decomposability, and association with fungi), abundance, and spatial arrangement of peatland plants affects the carbon balance of peatlands from a local to ecosystem scale [Limpens et al. 2008]. Peatlands also serve as long-term sinks for protons, nitrate, and sulfate [Alewell & Giesemann 1996, Küsel & Alewell 2004] and are therefore important barriers between agriculturally used land and surface waters. The large pore structure of peat creates a high water storage capacity [Boelter 1967] and plays an important role in local water balance and limiting water table fluctuations to the surface [Price 1996].

The predominantly water saturated soils present in peatland ecosystems [Clymo 1984] have the potential to act as a significant source of the greenhouse gas methane (CH_4) [Gorham 1991] and dissolved carbon (DC) to surface waters [Urban et al. 1989]. Emissions of CH_4 are estimated to release 46,000 tons of carbon annually, contributing 3-7% to the global atmospheric CH_4 deposition [Aselmann & Crutzen 1989]. Measured emission rates are high in peatlands, although emission rates vary spatially and temporally within peatland sites [Moore et al. 1990, Nilsson & Bohlin 1993].

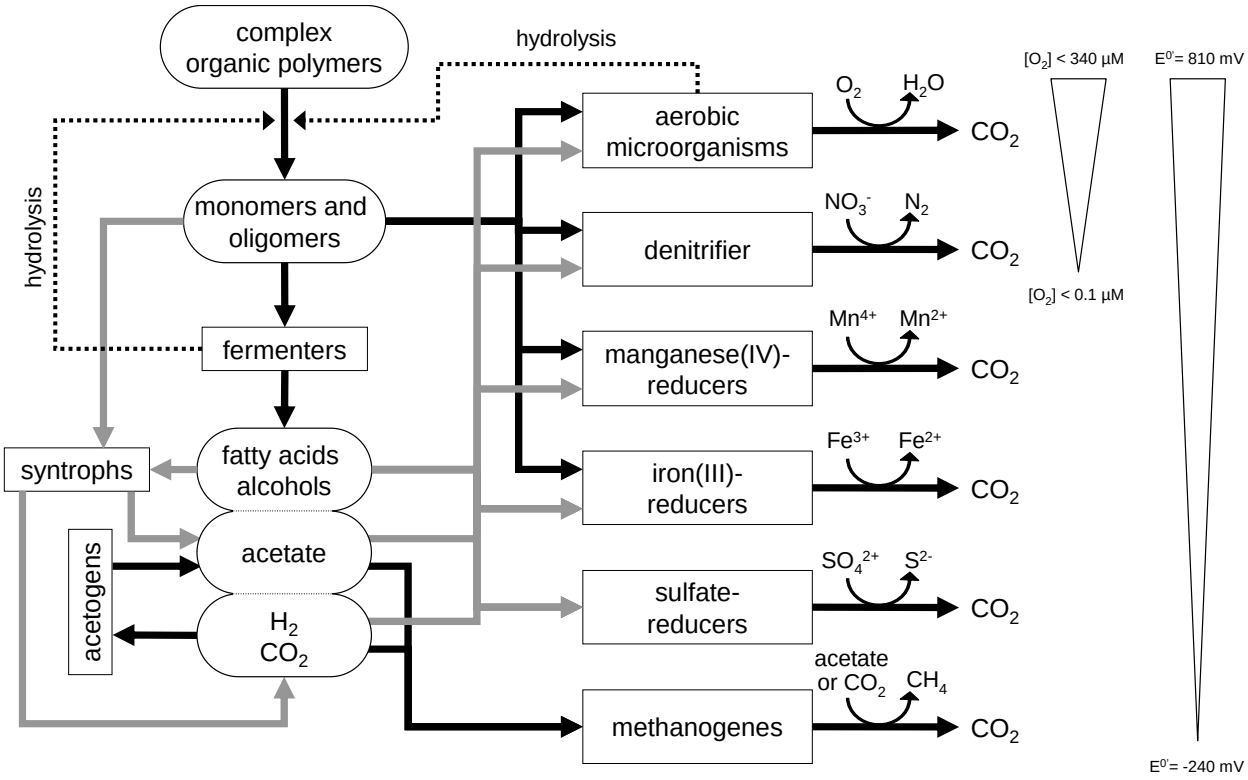

Figure I. Simplified pathway of organic matter degradation in peatlands with oxygen and redox gradients (according to Conrad [1999] and Westermann [1993]). $E^{0'}$: redox potentials determined at standard conditions; Squares: microbial functional groups; Ovals: carbon-intermediates resulting from microbial degradation; grey/black arrows: pathways of carbon and electron flow (note: different colors are for better visualization); dotted line: release of exoenzymes for hydrolysis of polymeric carbon compounds

Organic matter decomposition and C turnover in peatlands

The activity of extracellular enzymes, which are located outside of microbial cells [Chròst 1991], are important controls on the decomposition of complex organic matter within peat [Limpens et al. 2008]. Extracellular enzymes degrade complex polymers, such as polysaccharides, lipids, and proteins, into their corresponding monomers, such as sugars, fatty acids, and amino acids (Figure I). Often these resulting monomers can only be utilized by microorganisms and are therefore only available for microbial metabolism. According to the enzymatic latch hypothesis, exoenzymes can be inactivated or inhibited by phenolic compounds, such as humic substances. Biodegradation in peatlands appears to be reduced due to the presence of these compounds [Freeman et al. 2001], however, phenoloxidases may compensate by degrading the phenolic compounds (Pind et al., 1994). The absence of oxygen in water saturated peat should prevent the elimination of phenolic compounds by phenol oxidases [Freeman et al. 2001]. Hydrolases, i.e. β-D-glucosidases and phosphatases, are another important group of enzymes that mediate decomposition reactions from particulate

organic matter (POM) to dissolved organic matter (DOM). DOM is used for microbial metabolisms, such as fermentation. Primary fermentation results in the accumulation of a wide range of intermediates, such as acetate, propionate, lactate, alcohols, and hydrogen (H_2). These intermediates are then processed further during secondary fermentation or reduction of electron acceptors others than oxygen and finally release carbon as CO_2 or CH_4 (Figure I).

Decomposition processes in peatlands are generally slow compared to other ecosystems [Blodau 2002], however, microbial activity is known to be spatially diverse. In particular, rates of microbial respiration and exoenzymatic activity reach the highest activity in shallow peat zones (the first few cm) compared with deeper zones [Freeman et al. 1995, van den Pol-van Dasselaar & Oenema 1999] and the highest concentrations of CH_4 is found in the deeper anoxic zones [Hornibrook et al. 1997, Blodau et al. 2004].

The two main factors that limit decomposition rates are unfavorable environmental conditions for microorganisms involved in mineralization, i.e. low temperature, low pH, waterlogging, low availability of oxygen and the presence of humic substances, and the low availability of resources, due to the complexity of organic matter and low input of nutrients [Laiho 2006]. Thus, although peat soils represent a large C-pool [Gorham 1991], it was shown that the reduced quality of organic matter limits microbial metabolism because organic carbon is not bioavailable [Bridgham & Richardson 1992, Wagner et al. 2005]. Concentrations of CO_2 and CH_4 in peat profiles are therefore not only correlated with depth but also with botanical composition and/or the amount of peat decomposition [Nilsson & Bohlin 1993, Moore & Dalva 1997, Moore et al. 2007]. For example, peat dominated by *Carex* spp. contains lower amounts of cellulose and hemicellulose compared to *Sphagnum* spp. peats [Bohlin et al. 1989]. Both types of C-based compounds are likely substrates for hydrolytic fermentation [Zeikus 1983] and yield different amounts of precursors available for anaerobic CO_2 formation and methanogenesis. As a result, decomposition rates are lower for nonvascular species like *Sphagnum* spp. than for vascular species like *Carex* spp. (Verhoeven & Toth, 1995).

In general, labile metabolic compounds, such as sugars and amino acids, are available to microorganisms and are degraded prior to moderately labile structural compounds, such as cellulose and hemicellulose. Recalcitrant structural material, such as waxes, polyphenolics, lignin, cutin, are poorly degraded and remain in the system [Minderma 1968, Rubino et al. 2007]. Several studies have shown that aliphatic biopolymers are highly resistant to biodegradation in the waterlogged, anoxic conditions of peatlands and can be well preserved in soils [Winkler et al. 2005, Otto & Simpson 2006]. The byproduct of organic matter

degradation by microorganisms contributes to the POM pool and in the past, it was proposed to use quality indexes to estimate the degradability of organic matter. These indexes were based on the ratios of C to nitrogen (N), lignin to N, thermal degradability, infrared spectra, or chemical characteristics [Taylor et al. 1989, Gholz et al. 2000, Moore et al. 2007, Rubino et al. 2007, Artz et al. 2008, Rovira et al. 2008]. Currently, there is no common definition or a widely accepted quantitative index of organic matter "quality" [Rubino et al. 2007]. Therefore, a general definition that organic matter high in quality has a fast decay rate is not sufficient to describe the potential microbial activities in a given composition of peat.

Iron cycling in peatlands

Iron is the most abundant metal and the fourth most abundant element on earth and is omnipresent in the hydrosphere, lithosphere, biosphere and atmosphere [Kappler & Straub 2005]. It has been proposed that life emerged on a hot (up to 140–150°C), Fe(II)-rich early Earth 3.8 billion years ago [Gold 1992], where the abiotic photochemical generation of Fe(III) and H_2 provided early life an electron acceptor and energy source, respectively. As such, iron respiration has been proposed as one of the first forms of microbial metabolism [Vargas et al. 1998]. Currently, Fe(III) exists predominantly in the solid phase as oxyhydroxide minerals, e.g., Fe(III) oxides, at circumneutral pH, it is more soluble under acidic conditions [Lovley et al. 2004].

In general, poorly crystalline amorphous Fe(III) oxides, such as ferrihydrite, readily function as electron acceptors for Fe(III)-reducing prokaryotes (FeRP) [Lovley et al. 2004]. Fe(III) oxides predominantly exist in a crystalline phase or as a structural component of clays in modern soils and sediments and is less available for microbial activity [Roden 2003]. The thermodynamic favorability of crystalline Fe(III) oxide reduction, i.e., reduction of goethite, hematite, and magnetite, is lower compared to reduction of amorphous Fe(III) oxides, previous studies have shown that various strategies are used by microorganisms to increase the transfer of electrons to extracellular Fe(III). For example, humic acids, plant exudates, and microbially secreted compounds can be used as electron shuttles and organic ligands [Weber et al. 2006]. In addition, FeRP are known to use one or more alternative electron acceptors [Lovley et al. 2004], which might be advantageous in upper peat layers that experience varied redox conditions due to watertable fluctuations or oxygen release by plant roots.

The majority of cultured Fe(III)-reducing prokaryotes (FeRP) are neutrophilic and have only negligible capacities to reduce Fe(III) under moderately acidic (pH 3–6) conditions. Generally, there is a marginal understanding of the flow of carbon and reductants in acidic,

Fe(III) rich habitats and the related FeRP communities [Straub et al. 2001]. Numerous archaeal and bacterial genera of FeRP have been identified and these FeRP have diverse metabolic adaptations [Weber et al. 2006].

In minerotrophic fens, which are connected to iron-rich groundwater flow, Fe(III) can be an dominant electron acceptor for the mineralization of carbon. Under acidic pH conditions reduced Fe(II) persists for a long period of time, even in the presence of high O_2 levels and microbial oxidation becomes increasingly important [Kappler & Straub 2005]. In contrast, at near neutral or alkaline pH Fe(II) is readily chemically oxidized to Fe(III) with a half life in the order of several minutes in the presence of O_2 [Stumm & Morgan 1996]. Despite this rapid abiotic oxidation, aerobic, neutrophilic Fe(II)-oxidizing prokaryotes (FeOP) successfully compete for available Fe(II) [Kappler & Straub 2005]. The resulting amorphous Fe(III) oxides are high in reactive surfaces and thus, excellent substrates for FeRP [Emerson & Revsbech 1994, Roden & Zachara 1996]. Thus, FeOP likely play a significant role in Fe(II) oxidation in environments with distinct redox interfaces, such as those where diffusion limited O_2 transport leads to low dissolved O_2 partial pressure within the zone where Fe(II) and O_2 overlap. These environments are characterized by opposing diffusion gradients of O_2 and Fe(II) and are found in surface peat layers or in the rhizosphere of growing vegetation [Neubauer et al. 2007].

Competing electron accepting processes in peatlands

Anaerobic processes usually occur in the few centimeters below the soil surface where electron acceptors other than oxygen have to be used. The sequence of declining redox potentials (usually from +810 to -240 mV) [Ponnamperuma 1972] frequently begins with the reduction of nitrate to nitrite [NO_3^-/NO_2^-], manganese(IV) to manganese(II) [Mn(IV)/Mn(II)], ferric iron(III) to ferrous iron(II) [Fe(III)/Fe(II)], sulfate to sulfide [SO_4^{2-}/S^{2-}], and finally CO_2 reduction or acetate disproportionation yielding CH_4 (Figure I). Due to small spatial heterogeneity of soil conditions, these processes often overlap in space and time [Alewell et al. 2006].

Denitrification, the reduction of nitrate to nitrite, is typically limited in peatlands due to low NO_3^- concentrations caused by elution processes. In contrast, dissimilatory reduction of sulfate is an ongoing process in acidic seeps and fens, such as those located in the Lehstenbach catchment area [Alewell & Giesemann 1996, Alewell & Novak 2001]. This region received high atmospheric deposition of sulfur for decades due to the combustion of soft coal in Eastern Europe. As fens in the Lehstenbach region receive water not only from

precipitation, desorbed sulfate is transported from upland soils into the fens via groundwater flow. As oxidized and reduced sulfur species can be trapped by metals and organic matter, their amount in soils can be preserved from elution [Alewell & Giesemann 1996]. In fens, up to 70% of the impacted acidity can be neutralized in forested wetlands by sulfate- and Fe(III)-reduction processes [Sahin et al. 1998].

Methane is an important product of peat degradation and is mainly produced by strictly anaerobic methanogens either through the conversion of H_2 and CO_2 (hydrogenotrophic) or acetate (acetoclastic) (Figure I). Typically two-thirds of biogenically produced CH_4 in wetlands originates from acetoclastic methanogenesis [Conrad 1999], however, its proportion in peatlands is more varied. Evidence from Siberian wetlands shows the dominance of acetoclastic methanogenesis [Kotsyurbenko et al. 2004, Metje & Frenzel 2007], however, in northern peatlands, methanogenesis appears to be based on the conversion of H_2/CO_2 [Landsdown et al. 1992, Galand et al. 2005]. The competition of methanogens with other microorganisms, i.e., FeRP and sulfate reducing prokarytes (SRP), for electron donors can repress the production of CH_4 [Freeman et al. 1994, Kotsyurbenko et al. 2001, Roden & Wetzel 2003]. Therefore, sufficient amounts of SO_4^{2-} and Fe(III) as well as organic electron acceptors can depress CH_4 fluxes from peatlands [Achtnich et al. 1995, Nedwell & Watson 1995]. Despite this prediction, the addition of alternative electron acceptors did not always inhibit CH_4 production [Blodau & Moore 2003, Dettling et al. 2006]. In addition, it was also shown that methanogens may transfer electrons to Fe(III) [Bond & Lovley 2002, van Bodegom et al. 2004]. CH_4 also serves as substrate for methanotrophs which can oxidize CH_4 to CO_2 under oxic conditions [Segers 1998], leading to lower net emissions of CH_4 from peatlands compared with total methanogenesis.

Peatlands under future climate change

Today, the discussion of man-made global climate change and its potential impact on all ecosystems has increased. Peatlands, which cover less than 3% (~350 million ha) of the terrestrial land surface [Moore 2002], have attracted much attention, as they currently store approximately one-third of the terrestrial soil C pool (~ 455 billion tons of C) [Gorham 1991]. Climate models predict a dramatic decrease in annual precipitation in most European regions during the next decades [IPCC 2007]. However, northern Europe and other regions of the earth will likely receive increased precipitation combined with longer dry periods between rainfall events and more frequent summer droughts for central Europe [IPCC 2007].

The present global climatic warming in conjunction with a decrease in the annual rainfall will result in a decrease in the water table levels of boreal fens by 14-22 cm [Roulet et al. 1992a].

Numerous studies have demonstrated that a change in the water table level and temperature will directly affect carbon mineralization in peatlands [Moore & Dalva 1993, Metje & Frenzel 2005, Strack & Waddington 2007]. Thus, according to the enzymatic latch hypothesis, increased peat aeration, as a result of drought events could eliminate the critical mechanism restricting the rerelease of CO_2 to the atmosphere [Freeman et al. 2001]. However, this proposed increase in phenoloxidase activity could not be confirmed in general [Freeman et al. 1996, Williams 2000] and these studies dealing with exoenzymatic activities under altered hydrological conditions lead to conflicting results. Normally hydrolytic activities increase with a lowered water table [Freeman et al. 1996, Wang & Lu 2006, Song et al. 2007], but decreased activities have also been reported [Wang & Lu 2006]. Rewetting on the other hand can result in both the increase and decrease of enzymatic activity [Pulford & Tabatabai 1988, Corstanje & Reddy 2004, Wang & Lu 2006].

In water saturated peatlands anoxic conditions prevent the huge C pool of being released into the atmosphere and thus, CO_2 emissions will increase if the water table is lowered [Moore & Dalva 1993, Blodau et al. 2004, Jaatinen et al. 2008]. However, a water table decrease of about 5 cm has been shown to result in unchanging emissions of CO_2 [Freeman et al. 1996]. Rewetting of formerly dried terrestrial soils was reported to lead to a distinct CO_2 flush [Kieft et al. 1987, Fierer & Schimel 2003, Iovieno & Baath 2008] and was attributed to the release of physically protected organic matter. This release was due to disruption of soil aggregates or dissolution from soil surfaces [Appel 1998, Denef et al. 2001], the decomposition of dead microbial biomass killed by drying [Bottner 1985], and the release of carbon substrates by microbial hyposmotic stress responses [Kieft et al. 1987, Fierer & Schimel 2003]. In contrast, formation of CH_4 occurs in opposition to CO_2 with decreased or increased emissions at lower and higher water table levels, respectively [Freeman et al. 1993b, Moore & Dalva 1993, Blodau et al. 2004]. As the climate change in northern latitudes, there is a potential for peatlands to release stored C, as CO_2 and CH_4, to the atmosphere, creating a positive feedback with anthropogenic increases in greenhouse gas emissions [Bridgham et al. 1995, Gorham 1995]. During the last decades, atmospheric concentrations of the greenhouse gases, CO_2 and CH_4, have increased rapidly [Houghton 2005]. Of primary concern is the ability of CH_4 molecules to absorb infrared radiation, which makes it 20-30 times more efficient than CO_2 as a greenhouse gas [Srivastava 1998], and

results in a significant contribution to the radiative forcing of the atmosphere and global climate changes [IPCC 2007].

Induced periods of long and extensive peat oxygenation lead to the reoxidation of formerly reduced compounds to nitrate, Fe(III), and sulfate [Regina et al. 1996, Devito & Hill 1999, Paul et al. 2006], which are then available as electron acceptors after oxygen has been depleted. Thus, oxidation of upper soil layers may divert the flow of reductants from CH_4 formation to other electron accepting processes. Boreal peatlands will be affected by changes in biogeochemical cycles, productivity, and community composition as their ecosystem processes are controlled mainly by hydrology [Gorham 1991].

Thus, the following hypotheses were tested:

I. Oxygenation of peat increases the extent of potential Fe(III) reduction and enhances the microbial cycling of iron.

II. Oxygenation of peat leads to the activation of phenoloxidases and an increase in exoenzymatic activities and CO_2 formation rates.

III. A high proportion of thermal labile carbon compounds in peat is responsible for high rates of potential CO_2 and CH_4 formation.

PROJECT BACKGROUND AND AIMS

This thesis is part of the Research Unit 562 "Dynamics of soil processes under extreme meteorological boundary conditions" supported by the German Research Foundation (DFG). The background for this project is that global climate change scenarios predict a higher frequency of drying/rewetting cycles in soils, which should occur irregularly and with varying intensity in the future. These changes will influence the turnover of C, N, Fe, and S in soils to a large extent. Our knowledge of the resulting effects on underlying mechanisms for soil processes is limited the more general goals of the **DFG Research Unit 562** are to investigate:

- the effect and consequences of drying/wetting cycles on the turnover and fluxes of elements in an forested upland soil and an acidic peatland
- the physical, biological and chemical mechanisms that cause these effects

Thus, seven subprojects of the Research Unit 562 investigate (1) the redox cycling of carbon, sulfur, and iron, (2) the microbial cycling of carbon and iron, (3) the emissions of biogenic trace gases, (4) the dynamics of soil solution and solid phases, (5) the reaction of flow systems in the soil, (6) the growth of roots, and (7) the export of substrates and nutrients. Field experiments have been carried out at a forested upland soil and at an acidic peatland. As part of subproject 2, the main aim of **this thesis** was to investigate microbial mineralization processes influenced by water table changes and peat quality in the acidic peatland with respect to Fe(III) reduction.

The acidic fen "Schlöppnerbrunnen" is located in the Lehstenbach catchment area in the northern Fichtelgebirge, east central Germany (fen area: 0.8 ha, 50°07'55''N, 11°52'52''E) (Figure II A). There, the soil is Histosol on granite bedrock, and the vegetation is dominated by *Carex canescens, Carex rostrata, Juncus effuses, Molinia caerulea* and *Eriophorum vaginatum*.

Six different plots (7.2 m x 5 m each) were used for water table drawdown and rewetting experiments (Figure II B, C). The groundwater flows through the peat from the north to the south, and plots showed a water saturation gradient from east to west due to a slight slope in the field site. Water table drawdown was induced at three plots by installing roofs (Figure II D) and by additional pumping of groundwater from installed drainage tiles.

At the end of the manipulation experiments, the three plots were rewetted by irrigation of artificial rainwater.

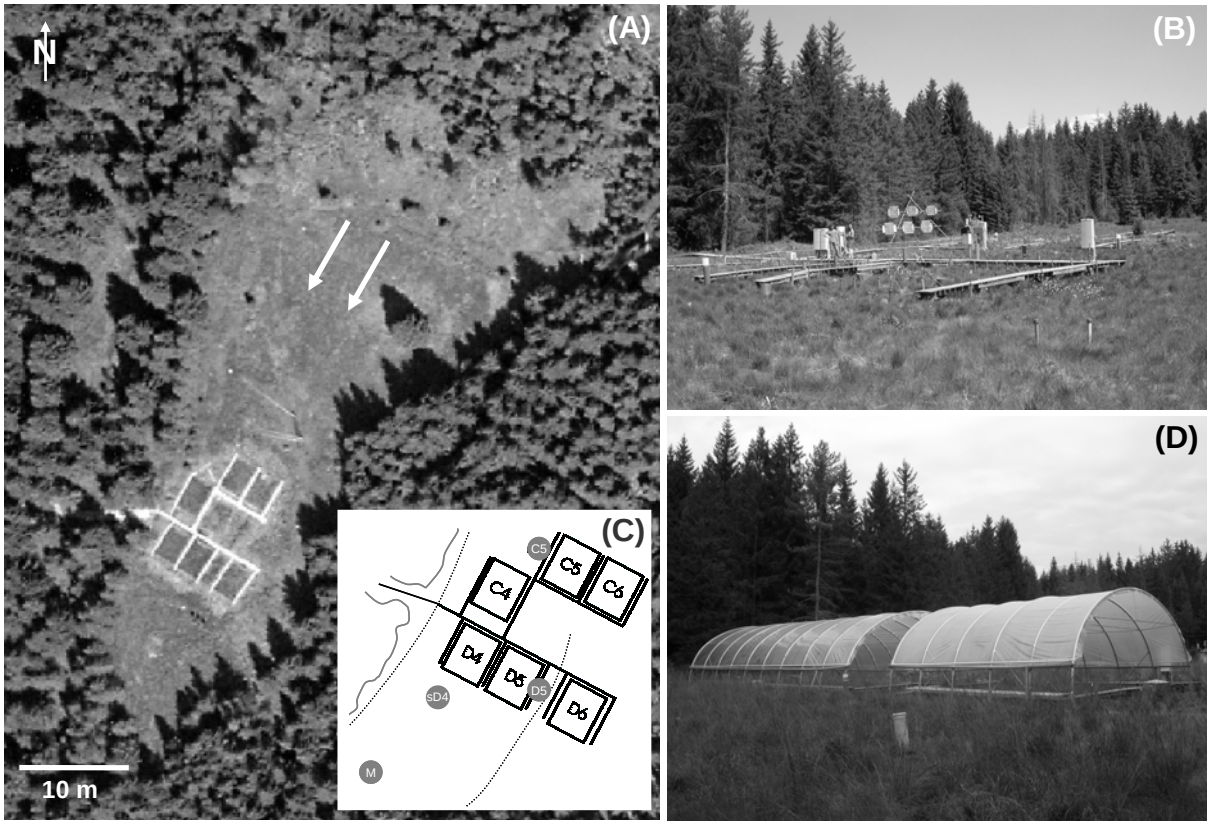

Figure II: Acidic fen "Schlöppnerbrunnen" in northern Bavaria, Germany. Air photograph (A; provided by Bayerische Vermessungsverwaltung, 2006), experimental plots at the fen site (B), detailed scheme of the control plots (C4-C6), manipulation plots (D4-D6) and the more southern sampling areas sD4 and M (C); Water table drawdown was experimentally initiated by roof construction (D). Arrows indicate the groundwater flow direction, the dotted lines unused drainage trenches, and the grayish line the flanking spruce forest.

Relevant microbial redox processes in fens and seeps of the Lehstenbach catchment area were described previously [Küsel et al. 2008] (added to the appendix section) and used as a basis for the three main chapters of this thesis. Fe(III) reduction was identified as a dominating process, especially in the iron-rich Schlöppnerbrunnen fen. I was involved in the phylogenetic characterization of Fe(III)-reducing microorganisms obtained from the upper peat zone during the beginning of my thesis. Based on this work, a more detailed characterization of the Fe(III)-reducing microbial community over depth was performed and discussed in the "General Discussion" part of this thesis. Furthermore, methanogenesis was also identified as a second important process in the catchment area interacting with Fe(III) reducing activities.

THESIS STRUCTURE

Chapter one is dealing with general aspects of competing Fe(III) reducing and methanogenic activities at the fen site during an annual season. We tested if seasonal hydrological fluctuations and shifts in vegetation activity during the year affect Fe(III) reduction and formation of CH_4 to get a better understanding of both processes at the field site. We determined the amount of total iron and investigated the microbial availability of Fe(III) over depth using artificial ligands and an electron shuttle. In particular, we determined the proportion of methanogenic pathways, to investigate the spatial distribution of methanogenic activities.

The effects of extreme weather conditions on mineralization processes in the fen are discussed in the **second chapter**. We determined the penetration depth of oxygen during water table drawdown and after rewetting, and tested if exoenzymatic activities, especially phenoloxidases, respond to the oxygenation of peat. The change of anaerobic to aerobic metabolism and the reoxidation of alternative electron acceptors would be favorable for microbial processes and thus, we followed the formation of Fe(II), CO_2, and CH_4 during the drying and rewetting cycle. In addition to the results presented in this chapter, other results obtained from the field manipulations were combined with data from the subproject 1 of the Research Unit 562 in an additional manuscript (see Knorr et al. in the "hervorgegangene und geplante Publikationen" section).

As a conclusion of the numerous anoxic peat incubations the peat quality was suggested to be a main important factor controlling the heterogeneity of CO_2 and CH_4 production activities at the field site. Thus, the aim of the **third chapter** was to derive a quality index based on thermo-degradability properties of peat to understand the effect of the chemical peat composition on the extent of anaerobic CO_2 and CH_4 forming processes on a spatial scale.

The "General Discussion" section was completed with additional information about the microbial cycling of iron in this fen. The role of Fe(II)-oxidizing microorganisms was investigated in an associated diploma thesis by Claudia Lüdecke and some of these results are highlighted (see also Lüdecke et al. in the "hervorgegangene und geplante Publikationen" section). Furthermore, a detailed phylogenetic characterization of selected Fe(III) reducing microorganisms over depth is described.

COMPETITION OF FE(III)-REDUCTION AND METHANOGENESIS IN AN ACIDIC FEN

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Abstract

Peatlands are sources of relevant green house gases like CH_4, but the temporal presence of Fe(III) may inhibit methanogenesis. Since excess of carbon during the vegetation period might allow concomitant electron-accepting processes, Fe(III)-reduction and methanogenesis were studied during an annual season in an acidic fen. Upper peat layer displayed highest Fe(II)- and CH_4 forming activities. Rates of Fe(II) formation did not change during the year and methanogenesis started mostly when Fe(II) formation reached a plateau. Most of the Fe(III) pool seemed to be bioavailable and addition of NTA stimulated only lightly Fe(II) formation, whereas EDTA and AQDS had no effect. In the presence of an inhibitor for methanogenesis (BES) Fe(II) formation was inhibited to 45%. Addition of Fe(III) during ongoing methanogenesis lead only to a partial inhibition of CH_4 formation. Proportion of acetoclastic methanogenesis varied between 42 and 90%, but no trend with time was observed. Numbers of acetate-, ethanol-, or lactate utilizing Fe(III)-reducers approximated 10^5-10^6 cells g (fresh wt peat)$^{-1}$. Fermentative glucose-utilizing Fe(III)-reducers were most abundant. Our results suggest that (i) methanogens used Fe(III) as electron acceptor and (ii) fermenting bacteria which do not compete with methanogens for common electron donors dominated the reduction of Fe(III) in this fen.

Keywords: Fe(III) reduction, peat, acetoclastic methanogenesis, co-activity

RESEARCH ARTICLE

Competition of Fe(III) reduction and methanogenesis in an acidic fen

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Abstract

Peatlands are sources of relevant greenhouse gases such as CH_4, but the temporal presence of Fe(III) may inhibit methanogenesis. Because excess of carbon during the vegetation period might allow concomitant electron-accepting processes, Fe(III) reduction and methanogenesis were studied during an annual season in an acidic fen. The upper peat layer displayed the highest Fe(II)- and CH_4-forming activities. The rates of Fe(II) formation did not change during the year and methanogenesis started mostly when Fe(II) formation reached a plateau. Most of the Fe(III) pool seemed to be bioavailable, and addition of nitrilotriacetic acid stimulated only light Fe(II) formation, whereas EDTA and anthraquinone-2,6-disulfonate had no effect. In the presence of an inhibitor for methanogenesis (sodium 2-bromoethanesulfonate), Fe(II) formation was inhibited to 45%. Addition of Fe(III) during ongoing methanogenesis led only to a partial inhibition of CH_4 formation. The proportion of acetoclastic methanogenesis varied between 42% and 90%, but no trend with time was observed. The number of acetate-, ethanol- or lactate-utilizing Fe(III) reducers approximated 10^5–10^6 cells g (fresh wt peat)$^{-1}$. Fermentative glucose-utilizing Fe(III)-reducers were most abundant. Our results suggest that (1) methanogens used Fe(III) as an electron acceptor and (2) fermenting bacteria, which do not compete with methanogens for common electron donors, dominated the reduction of Fe(III) in this fen.

Introduction

Peatlands are wetland ecosystems and release the greenhouse gas methane (Aselmann & Crutzen, 1989; Fung *et al.*, 1991). They cover $< 3\%$ of the Earth's terrestrial surface (Clymo, 1987), but store *c.* 30% of global soil carbon. Although two-thirds of the methane formed in wetlands originate from acetate (Conrad, 1999), the proportion of acetoclastic and hydrogenotrophic methanogenesis can deviate in peatlands. In most northern peatlands, methanogenesis is based on H_2–CO_2 (Williams & Crawford, 1984; Landsdown *et al.*, 1992; Horn *et al.*, 2003). However, from Siberian wetlands comes evidence for acetoclastic methanogenesis (Kotsyurbenko *et al.*, 2004; Galand *et al.*, 2005; Metje & Frenzel, 2005, 2007). Methanogenesis can shift from acetoclastic methanogenesis in the upper vegetated zone toward the reduction of CO_2 in deeper zones (Svensson, 1984; Popp *et al.*, 1999; Chasar *et al.*, 2000). The accumulation of acetate in some peatlands as a terminal product of anaerobic decomposition indicates that it is not the primary source of CH_4 formation (Hines *et al.*, 2001; Duddleston *et al.*, 2002; Rooney-Varga *et al.*, 2007).

Water table fluctuations allow the penetration of oxygen into the soil leading to reoxidation of the pool of reduced compounds such as humic substances, reduced sulfur species and Fe(II) in peatlands. Anthropogenic sulfate depositions lead to increased sulfate-reducing activities in many northern peatlands (Alewell & Giesemann, 1996; Vile *et al.*, 2003; Loy *et al.*, 2004). In contrast to ombrotrophic bogs, concentrations of iron can be enhanced in fens, which are subjected to external flows (Mitsch & Gosselink, 2000). Vegetation may enhance Fe cycling through the leakage of O_2 from living plant roots, which is an important mechanism for regenerating reactive Fe oxides in anoxic habitats (Kostka & Luther, 1995; Mendelssohn *et al.*, 1995; Roden & Wetzel, 1996; Frenzel *et al.*, 1999). Roots excrete easily available carbon substrates such as organic acids, alcohols and sugars. These compounds might not only fuel Fe(III) reduction as a carbon source but also enhance the availability of Fe(III) by serving as organic chelators that maintain Fe(III) in a soluble form

(Luther *et al.*, 1992), and allow a contact with microbial cells in surrounding anoxic microzones (Lovley & Woodward, 1996). The high concentration of humic substances in peatlands could also be advantageous for Fe(III) reduction because of their potential to act as electron shuttles (Lovley *et al.*, 1998). Suppression of methanogenesis by Fe(III) reduction is explained by thermodynamic theory, which predicts that the energetically more favorable electron acceptor will be utilized first under substrate-limited conditions (Ponnamperuma, 1972; Zehnder & Stumm, 1988). Fe(III)-reducing microorganisms (FeRB) are able to utilize acetate and H_2 at concentrations far below levels that can be metabolized by methanogens. The half-saturation constants (K_m) determined for acetate uptake and endogenous acetate concentrations are much lower in Fe(III)-reducing sediments than those in methanogenic slurries (Roden & Wetzel, 2003). In addition, methanogens may transfer electrons to Fe(III) (Bond & Lovley, 2002), suggesting that factors other than substrate competition play a role in inhibition of methanogenesis during Fe(III) reduction.

Recent studies have shown that the reduction of Fe(III) is an important alternative electron-accepting process in methane-emitting acidic fens in the Lehstenbach catchments that receive iron with the groundwater flow (Küsel & Alewell, 2004). With this project we want to study in more detail the competing Fe(III)-reducing and methanogenic activities in the fen, which might change due to water table fluctuations and shifts in vegetation activity during the year, and enumerate the abundances of FeRB with respect to their metabolic types.

Materials and methods

Field site

Samples were obtained from an acidic fen in the northern Fichtelgebirge in east-central Germany (fen area: 0.8 ha, 50°07′55″N, 11°52′52″E). The soil is Histosol on granite bedrock, and the vegetation is dominated by *Carex canescens*, *Carex rostrata*, *Juncus effuses, Molinia caerulea* and *Eriophorum vaginatum*. Peat accumulation ranged from 30 to 70 cm. Groundwater flows slightly through the peat from the north to the south (Paul *et al.*, 2006). The mean annual air temperature at this site approximates 6.5 °C with extremes of *c.* 30 and −20 °C and an annual precipitation average of 1100 mm. Soils were not frozen during the winter of 2005/06, but were covered with large amounts of snow from November to April, leading to a heavy snow-melting event at the end of April. The peat temperature ranged between 0.5 and 15 °C at 10-cm depth.

Peat and soil water sampling

Replicate peat cores were sampled in August and December 2005, April, June, August, September, October and November 2006, and June 2007 with a peat core (inner diameter, 8 cm) at a central part of the fen (C5). Additional samples were obtained 25 and 50 m south of C5 in November 2006. The water table at these sites was higher than at C5 and showed lower fluctuations. The degree of peat decomposition was determined after von Post's humification scale (Puustjärvi, 1970). Samples were taken from 0- to –40-cm depth and sectioned into four zones (I: 0–10; II: 10–20; III: 20–30; IV: 30–40 cm) and transported to the laboratory in airtight plastic bags at 4 °C in a temperature-insulated box. Samples were processed the same day or stored at 4 °C for some days. Field fresh duplicate peat samples were dried at 105 °C for 24 h to determine their dry weights. Soil organic matter (SOM) was analyzed by loss-on-ignition at 500 °C for 4 h. According to Zak & Gelbrecht (2007), total C, H, N and S of dried (60 °C for 48 h) and milled (centrifugal ball mill, pulverisette 6, Fritsch, Germany) soil samples were measured with an elemental analyzer (vario EL, Elementar), total Fe, Al, Ca and Mg were analyzed after reverse aqua regia decomposition by flame atomic absorption spectrometry (Perkin Elmer, 3300), and total P was measured photometrically (Varian, Cary 1E) after acid digestion by the molybdenum blue method (Murphy & Riley, 1962) (Table 1).

Soil water samples were obtained next to the cores obtained from C5 at the same time. Under waterlogged conditions, soil water samples were taken using the dialysis sampler technique (Hesslein, 1976; Steinmann & Shotyk, 1996) up to 40-cm depth in steps of 1 cm. The samplers (material: Perspex coated with stainless steel) were treated and exposed as described in detail by Zak *et al.* (2004). Alternatively, soil solution was gained with 10-mL disposable syringes (Terumo, Belgium) from prior installed Rhizon suction samplers (Eijkelkamp, the Netherlands) in 1-, 5-, 10-, 15-, 20-, 25-, 30-, 35- and 40-cm depths. Soil water was analyzed for Fe(II), Fe(III), NO_3^-, NH_4^+, SO_4^{2-}, PO_4^{3-}, pH and organic compounds in all samples and for CH_4 and CO_2 only in samples obtained with the dialysis sampler.

A modified method of Edenborn & Brickett (2001) and Paul *et al.* (2006) was used to evaluate the presence of oxygen in peat. Commercial polyvinyl chloride cable funnels (70 × 9 × 8 mm) were poured with melted 2% agar containing *c.* 80 mM particulate black FeS ($FeSO_4 \cdot 7\ H_2O$ mixed in a 1 : 1 ratio with $Na_2S \cdot nH_2O$, solved in deionized water). After solidifying, the FeS probes were wrapped in plastic film to avoid contact with oxygen. Nine, always newly prepared FeS probes were placed vertically in the fen around the sampling locations, but about 1 m apart to avoid Fe or SO_4^{2-} contamination of soil samples. After a minimum of 3 days, FeS probes were removed and the color was determined using the Munsell color chart and classified into two groups. A change in color from black to brownish caused by oxidized FeS to Fe(III)-oxyhydroxide indicated the presence

Table 1. Geochemical characteristics of the fen ($n = 2$)

Depth (zone) (cm)	pH*	P_{total} [µmol g (dry wt peat)$^{-1}$]	Fe_{total} [µmol g (dry wt peat)$^{-1}$]	Al_{total} [µmol g (dry wt peat)$^{-1}$]	Mg_{total} [µmol g (dry wt peat)$^{-1}$]	Ca_{total} [µmol g (dry wt peat)$^{-1}$]	DM (%)	SOM (%)	N_{total} (%)	C_{total} (%)	H_{total} (%)	S_{total} (%)	C/N
0–10 (I)	4.7	58.1	170.1	800.5	37.0	5.0	20	70	2.0	36.2	4.5	0.3	18.6
10–20 (II)	4.7	48.4	105.6	563.3	12.3	2.5	18	83	2.1	47.3	5.4	0.3	22.6
20–30 (III)	4.6	25.8	111.0	441.0	12.3	7.5	18	88	1.5	51.5	5.5	0.2	35.3
30–40 (IV)	4.8	32.3	57.3	600.4	20.6	5.0	14	87	1.2	50.4	6.0	0.2	40.6

*Soil water pH ($n = 18$).
DM, dry mass; SOM, soil organic matter.

of oxygen. In general, the change in color was very sharp, mostly within a few millimeters and only in some cases stretched over a few centimeters.

Peat microcosm studies

Peat was sectioned in four depth zones, and peat material from replicate cores was pooled. Twenty grams of fresh weight peat was placed in sterile 150-mL incubation flasks (Mueller & Krempel, Buelach, Switzerland) under a continuous flow of sterile argon. Flasks were closed with rubber stoppers and screw caps, and were incubated in the dark at 15 °C with an initial overpressure of *c.* 200 mbar. Methyl fluoride (CH_3F, final headspace concentration of 1%) was added as selective inhibitor to determine acetoclastic methanogenesis (Frenzel & Bosse, 1996; Janssen & Frenzel, 1997; Metje & Frenzel, 2007). A final concentration of 40 mM sodium 2-bromoethanesulfonate (BES) was used to completely inhibit methanogenesis. For gas analyses, headspace samples were obtained with sterile syringes at 10–20 individual time points. For sampling of the solid phase, three flasks were harvested after selective time intervals and used for extraction. Activity rates were calculated by linear regression analysis during the time period of linear increase of Fe(II) or CH_4. Approximately 2–3 µmol g (fresh wt peat)$^{-1}$ of the following electron donors – formate, acetate, ethanol or glucose, or a mixture of H_2 and CO_2, which equalled 24 and 8 µmol g (fresh wt peat)$^{-1}$, respectively – were added to peat microcosms from sterile anoxic stock solution or sterile gas. Similarly, chelating agents [EDTA or nitrilotriacetic acid (NTA), 5 µmol g (fresh wt peat)$^{-1}$ each] or the electron shuttling compound anthraquinone-2,6-disulfonate [AQDS, 100 nmol g (fresh wt peat)$^{-1}$] were added. To stimulate Fe(III) reduction in soil from 30- to 40-cm depth, peat was mixed first with an amorphic Fe(III) oxyhydroxide [$Fe(OH)_3$] suspension (Lovley & Phillips, 1986) in a bucket to obtain 30 µmol Fe(III) g (fresh wt peat)$^{-1}$ as an additional electron acceptor. To calculate the reducing equivalents theoretically obtained from supplemental substrate oxidation recovered in Fe(II), stoichiometries for Fe(III) reduction were used (e.g. complete oxidation of glucose: $C_6H_{12}O_6 + 24\text{-}Fe(III) + 24OH^- \rightarrow 6CO_2 + 24Fe(II) + 18H_2O$; efficiency of 100% is given if 24 mol Fe(III) is reduced to 24 mol Fe(II) coupled with the oxidation of 1 mol glucose to 6 mol CO_2).

To study the direct competition of Fe(III) reduction and CH_4 formation, 20 g fresh wt peat was mixed with 50 mL of a mineral solution [per liter: 5 mg $(NH_4)_2SO_4$, 5.4 mg Na_2SO_4, 4 mg $CaCl_2 \cdot 2H_2O$, 4 mg $MgCl_2 \cdot 6\ H_2O$, 0.2 mg KH_2PO_4 adjusted to pH 5, 10 mL trace metal solution (Drake, 1994), 1 mL vitamin B solution (Drake, 1994)]. After 26 days of incubation, $Fe(OH)_3$ suspensions or equivalent volumes of sterile deionized water were added to three replicate peat slurries during the phase of CH_4 formation to obtain additional 50 µmol Fe(III) g (fresh wt peat)$^{-1}$.

A *t*-test was used (SPSS 15.0, SPSS Inc., Chicago, IL) to verify whether the addition of chelating agents or electron donors significantly influenced the formation of Fe(II).

Enumeration of peat microorganisms

The number of FeRB was determined by the most probable number (MPN) technique (de Man, 1975). Tenfold serial dilutions of peat at 0–10-, 10–20- and 30–40-cm depth were prepared in sterile anoxic mineral solution (per liter: 200 mg KH_2PO_4, 10 mg $MgCl_2 \cdot 6H_2O$, 200 mg NaCl, 200 mg NH_4Cl, 10 mg $CaCl_2 \cdot 2H_2O$, pH 6). Anoxic growth media consisted of (per litre) 50 mg yeast extract, 2 g $(NH_4)_2SO_4$, 1 g KCl, 0.5 g $K_2HPO_4 \cdot 3H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 5 mL trace metal solution (Drake, 1994) and 5 mL vitamin B solution (Drake, 1994). As electron acceptor, either 40 mM $Fe(OH)_3$ or 40 mM Fe(III)-citrate was added. The pH was adjusted to 5.5 or 7.0 as indicated. Acetate, ethanol, glucose or lactate was added separately to reach a final concentration of 5 mM. BES (40 mM) was added to additional ethanol MPN incubations to inhibit syntrophic ethanol oxidation to acetate coupled to H_2 scavenging by methanogens. H_2 resulting from ethanol oxidation can be used to reduce Fe(III) by methanogens in acidic peat (Metje & Frenzel, 2005). Tubes were inoculated in triplicate and were incubated vertically at 15 °C in the dark. After 6 months of incubation, tubes were scored as positive for Fe(III) reduction if at least 400 μM of Fe(II) was produced. MPN values and 95% confidence limits were calculated from standard MPN tables (de Man, 1975, 1977).

Total bacteria in peat were enumerated by the 4′,6-diamidino-2-phenylindole (DAPI) method according to Porter & Feig (1980). Bacteria were removed from peat with a chemical and mechanical extraction following protocols from Mermillod-Blondin *et al.* (2001) and Lunau *et al.* (2005). Sterile extraction solution (10 mL) containing 5 mM $Na_4P_2O_7$, 70 mM NaCl, 5 mM EDTA and 10% methanol was added to 200 mg field fresh peat and incubated for 30 min at maximum intensity and 30 °C in an ultrasonic bath (Sonorex DK 514BP, 35 kHz, 2 × 450 W, Bandelin). After 30 min, a 1-mL aliquot of supernatant was fixed with 1 mL ethanol (98%), 40-fold diluted with sterile deionized water and stained with DAPI to a final concentration of 5 μg mL^{-1} (Schallenberg *et al.*, 1989). Cells were counted in duplicate to a minimum of 400 cells per filter with a Zeiss Axiolab microscope at × 1000 magnification (lamp: HBO 100; filter set: Zeiss, Ex 450-490, FT 510, LP 515).

Analytical techniques

Headspace gases were measured with Hewlett-Packard Co. 5980 series II gas chromatographs according to Küsel & Drake (1995). Gas pressures in flasks were measured with a TensioCheck TC 1066 (Tensio-Technik, Geisenheim, Germany) needle manometer. Dissolved CH_4 and CO_2 sampled with dialysis chambers were removed from the water phase by acidifying subsamples with 4 M HCl in closed vials. Concentrations of aliphatic fatty acids, sugars and alcohols were determined with a Hewlett-Packard 1090 series II HPLC equipped with an Aminex ion exclusion HPX-87H column (300 × 7.8 mm; Bio-Rad) and an ERC RI-101 refractive index detector (Riemerling, Germany). Samples were clarified by centrifugation (tabletop centrifuge TH21, VEB MLW, Engelsdorf, Germany) at 9000 ***g*** and by microfiltration with 0.2-μm nylon filters (Rotilabo, Karlsruhe, Germany) before HPLC analyses.

SO_4^{2-}, PO_4^{3-} and NO_3^- were analyzed by ion chromatography with a Dionex (Sunnyvale, CA) DX 120 ion chromatograph equipped with an IonPacAS14 column. Ammonia was measured spectrophotometrically (Uvikon 931, Kontron Instruments, Italy) by the hypochlorite–nitroprusside method (Harwood & Huyser, 1970). The rate of Fe(III) reduction was estimated by determining the amount of Fe(II) formed in the culture tubes or incubation flasks. Peat (three replicate microcosms at each time point) was extracted with 50 mL 0.5 M HCl, or aliquots of 0.2 mL of peat suspensions from microcosms or MPN tubes were taken with sterile N_2-flushed syringes, transferred to 9.8 mL of 0.5 M HCl and incubated for 1 h at room temperature. After centrifugation, HCl-extractable Fe(II) was measured spectrophotometrically (Uvikon 931, Kontron Instruments) following the phenanthroline method of Tamura *et al.* (1974). Initial experiments demonstrated that rates of Fe(II) formation from peat microcosms were identical to those of peat suspensions on a dry weight basis. Soil water pH was measured by a WTW pH Meter (pH 330, Weilheim, Germany) combined with an InLab 423 combination pH micro-electrode (Mettler Toledo, Giessen, Germany).

Results

Stimulation of microbial Fe(III) reduction

Spontaneous Fe(II) formation rates were highest in zone I (0–10 cm) and low to negligible below 20-cm depth (Fig. 1, Table 2). The addition of NTA caused a significant increase in the Fe(II) formation rate from 27.8 to 49.0 μmol Fe(II) g (dry wt peat)$^{-1}$ day^{-1} in zone I (Fig. 2a). The increase of the rate from 12.2 to 14.7 μmol Fe(II) g (dry wt peat)$^{-1}$ day^{-1} in zone II was not significant (Fig. 2b). Addition of AQDS and EDTA had no or even a slightly inhibitory effect. The extent of Fe(II) formation in zone II was increased in NTA treatments [112.4 μmol Fe(II) g (dry wt peat)$^{-1}$] but declined with additional EDTA [76.0 μmol Fe(II) g (dry wt peat)$^{-1}$] compared with the control [95.8 μmol Fe(II) g (dry wt peat)$^{-1}$]. The addition of glucose, ethanol and formate as supplemental

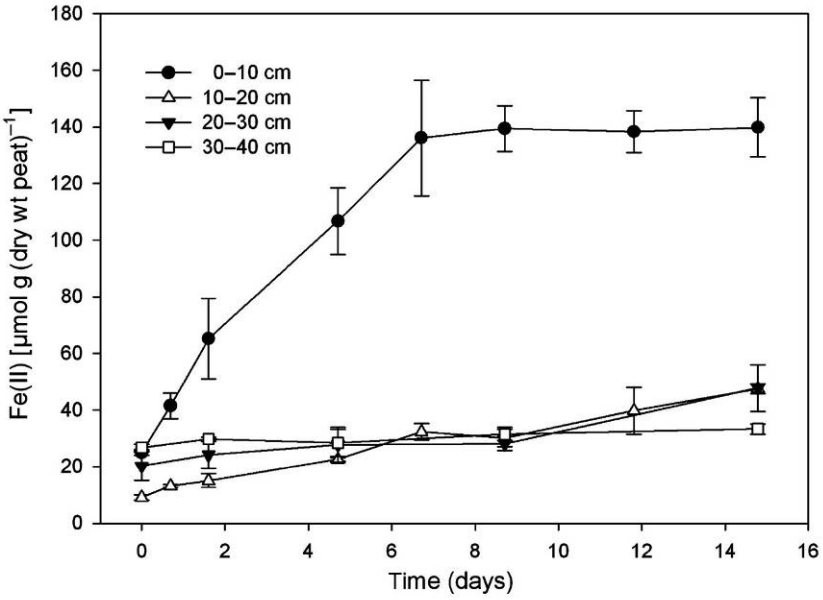

Fig. 1. Depth profile of Fe(II) formation ($n = 3$) in different depth zones (0–40 cm). Samples were obtained from C5 in August 2006.

Table 2. Initial Fe(II) concentrations, Fe(II) formation rates and final Fe(II) concentrations in microcosm experiments with peat obtained from different depth zones (0–40 cm; C5) ($n = 3$)

Depth (zone) (cm)	December 2005	April 2006	June 2006	August 2006	September 2006	October 2006	November 2006
Initial Fe(II) formation rates [µmol g (dry wt peat)$^{-1}$ day^{-1}]							
0–10 (I)	21.5	13.5	16.1	16.0	16.5	6.1	13.9
10–20 (II)	4.0	2.8	2.7	2.5	2.0	2.5	1.2
20–30 (III)	NA	3.4	−1.0	1.7	1.4	4.4	5.0
30–40 (IV)	0.1	0.4	0.0	0.1	0.0	0.4	−0.1
Initial Fe(II) concentrations [µmol g (dry wt peat)$^{-1}$]							
0–10 (I)	32.5	26.8	81.9	25.0	44.8	26.4	23.0
10–20 (II)	45.6	30.6	53.9	9.0	15.8	18.8	12.0
20–30 (III)	NA	68.3	80.5	20.2	38.5	29.0	10.7
30–40 (IV)	50.1	59.1	41.0	26.7	31.5	38.6	35.6
Final Fe(II) concentrations [µmol g (dry wt peat)$^{-1}$]							
0–10 (I)	165.0	160.6	149.7	139.8	182.9	130.1	129.7
10–20 (II)	70.7	71.7	110.0	47.6	57.3	70.7	74.0
20–30 (III)	NA	102.5	78.3	47.9	68.8	130.2	59.8
30–40 (IV)	52.2	65.0	43.8	33.6	35.3	49.6	40.4

NA, not analyzed.

electron donors stimulated the formation of Fe(II) significantly compared with the control (Table 3). In glucose-supplemented peat microcosms, glucose was totally consumed without apparent delay, and lactate and acetate were detected as fermentation products in zone I, and ethanol, lactate and acetate in zone IV. Twice as much reducing equivalents that were theoretically obtained from glucose oxidation were recovered in Fe(II) in zone I compared with zone IV. In ethanol-supplemented peat microcosms, ethanol consumption occurred during enhanced Fe(II) formation in zones I and IV. Small amounts of formate and acetate could be detected as fermentation products from ethanol in the first 2 days of incubation in zone I. Formate oxidation appeared to be totally coupled to Fe(II) formation in zone I, but only partially in zone IV. Supplemental acetate was consumed, but had only negligible effects on Fe(II) and CO_2 formation. Addition of H_2 and CO_2 stimulated Fe(II) formation also in deeper soil without the addition of amorphic $Fe(OH)_3$ suspension (Fig. 3). As a mean, 23% of the reducing equivalents from hydrogen oxidation were recovered in Fe(II).

Enumeration of FeRB

The number of enumerated FeRB decreased only slightly with increasing soil depth (Table 4). Lactate- and ethanol-utilizing FeRB were more abundant in all zones than acetate-

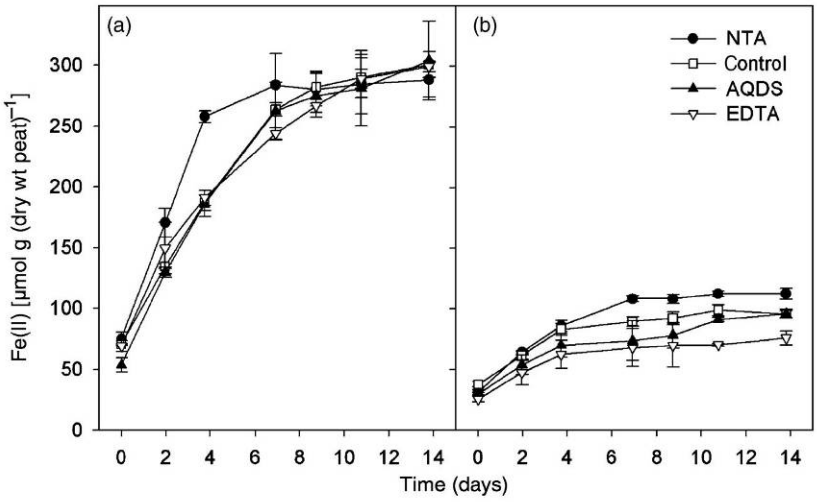

Fig. 2. Effect of electron shuttling compounds (AQDS) and chelating agents (EDTA, NTA) on Fe(II) formation ($n = 3$) in peat obtained from C5 in December 2005 (a: 0–10 cm; b: 10–20-cm depth). AQDS, humics analogue, anthraquinone–2,6-disulfonate; NTA, nitrilotriacetic acid; EDTA, ethylenediaminetetraacetic acid.

Table 3. Consumption of substrates and formation of Fe(II) and CO_2 after 9 days of incubation in anoxic peat microcosms ($n = 3$)

Depth (cm)	Substrate	Substrate added [µmol g (fresh wt peat)$^{-1}$]	Substrate consumption within 9 days (%)	Final concentration after 9 days [µmol g (fresh wt peat)$^{-1}$]		Recovery (%)	
				Fe(II)	CO_2	Fe(II)	CO_2
0–10	Glucose	2.0	100	32.0	14.5	19	39
	Ethanol	2.0	100	27.8	10.2	23	13
	Acetate	3.0	98	24.2	10.7	6	15
	Formate	2.5	100	28.0	11.7	114	83
	Control	NA	NA	22.6	9.7	NA	NA
30–40	Glucose	2.0	100	5.9	8.3	8	52
	Ethanol	2.0	78	4.7	3.7	9	34
	Acetate	3.0	69	2.6	4.1	1	31
	Formate	2.5	100	4.0	5.0	31	105
	Control	NA	NA	2.4	2.4	NA	NA

Recovery of carbon and reducing equivalents were calculated according to the theoretical complete oxidation of the substrates consumed. Peat was obtained from C5 in June 2006.
NA, not applicable.

utilizing FeRB. Surprisingly, MPN values of acetate treatments cultured at pH 7.0 were significantly lower than those obtained at pH 5.5. The number of ethanol-utilizing FeRB was significantly higher in the absence of BES. Ethanol consumption in MPN tubes not supplemented with BES yielded nearly equimolar amounts of acetate (5 mM), high amounts of Fe(II) (12 mM) and only trace amounts of CH_4. In the presence of BES, no acetate or CH_4 but small amounts of H_2 (up to 0.3 mM) were formed in the low dilutions in which ethanol was completely consumed and high amounts of Fe(II) were formed. Lactate consumption yielded acetate in all peat dilutions. Acetate was only partially consumed at pH 5.5 and pH 7 in MPN dilutions. The number of glucose-utilizing FeRB in Fe-citrate medium was most abundant, and 5–10% of the reducing equivalents from glucose oxidation were recovered in Fe(II). Glucose was fermented to acetate, ethanol, butyrate and formate in zone I, additionally to lactate in zone II, and to propionate in zone IV. The total number of DAPI-stained microorganisms in the peat approximated to $2.1–4.4 \times 10^9$ cells g (fresh wt peat)$^{-1}$ (Table 4).

Seasonal variations of Fe(II) formation

In general, water tables ranged from 0 to 10 cm below the surface. After the snow-melting event in April 2006, Fe(II) concentrations in soil water were highest in 0–5- and 35–40-cm depth with up to 240 µM (data not shown). SO_4^{2-} and NO_3^- were not detected. In July 2006, CH_4 reached 30 µM below 10-cm depth. However, during the hot summer, the water table decreased to 40 cm from August to September. Oxygen penetrated down to 34 cm in August 2006 as

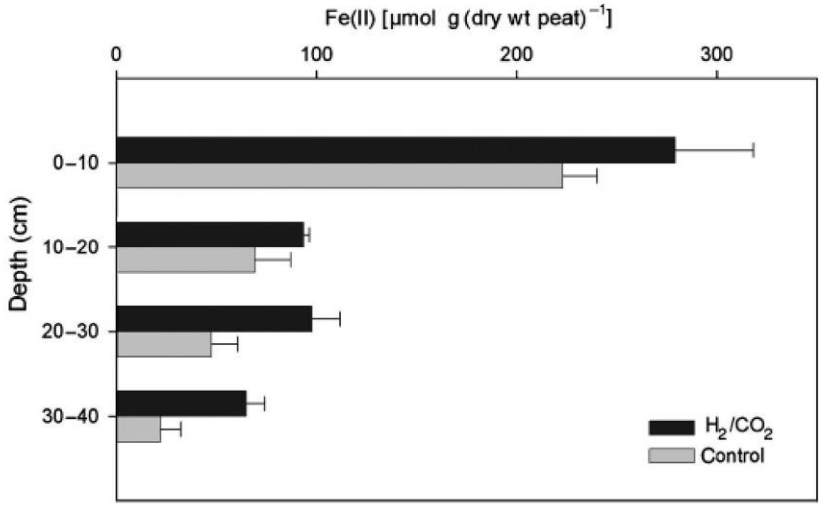

Fig. 3. Depth profile (0–40-cm depth) of final Fe(II) concentrations in peat microcosms with supplemented H_2/CO_2 compared with control ($n = 3$). Samples were obtained from C5 in June 2007.

Table 4. MPN values of Fe(III) reducers from three different depth zones ($n = 3$) in the fen observed at pH 5.5 or 7

			MPN [g (fresh wt peat)$^{-1}$] (95% confidence limit)*		
Electron donor	Electron acceptor	pH	0–10 cm	10–20 cm	30–40 cm
Acetate (5 mM)	$Fe(OH)_3$ (40 mM)	5.5	4.0×10^5 (0.9–19)[a]	9.0×10^3 (1.9–42)[b]	2.3×10^5 (0.5–11)[a]
Acetate (5 mM)	$Fe(OH)_3$(40 mM)	7	2.3×10^3 (0.5–11)[a]	4.0×10^3 (0.9–19)[a]	2.3×10^3 (0.5–11)[a]
Ethanol (5 mM)	$Fe(OH)_3$(40 mM)	5.5	4.0×10^6 (0.9–19)[a]	4.0×10^5 (0.9–19)[b]	4.0×10^5 (0.9–19)[b]
Ethanol (5 mM)[†]	$Fe(OH)_3$(40 mM)	5.5	2.3×10^5 (0.5–11)[a]	2.3×10^4 (0.5–11)[b]	2.3×10^4 (0.5–11)[b]
Lactate (5 mM)	$Fe(OH)_3$(40 mM)	5.5	4.0×10^6 (0.9–19)[a]	4.0×10^5 (0.9–19)[b]	4.0×10^5 (0.9–19)[b]
Glucose (5 mM)	Fe-citrate (20 mM)	5.5	9.0×10^6 (1.9–42)[a]	4.0×10^6 (0.9–19)[a]	2.3×10^6 (0.5–11)[a]
Total DAPI-stained cells	NA	NA	4.6×10^9 (3.7–5.6)	4.2×10^9 (3.5–4.9)	2.1×10^9 (1.6–2.5)

Peat was obtained from C5 in August 2005.
*95% confidence limit with the same exponent as the mean.
[†]Addition of 40 mM BES.
[a,b]Significant differences of the MPN values between the three depth zones for each supplemental electron donor. Significance is given when the ratios between MPNs is above 8.87 (Alef, 1991).
NA, not applicable.

indicated with the FeS probes. The pH approximated 4.3 during oxic conditions compared with 5.1 under more reduced conditions. From August till October, no Fe(II) but high nitrate (31 µM) and sulfate (180 µM) concentrations were detected in the soil water. Initial Fe(II) concentrations determined in the peat solid phase were small in the same period down to 40-cm depth (Table 2). Concentrations of alcohols, sugars and short chain fatty acids were below the detection limit of *c.* 30 µM.

Despite fluctuating redox conditions, Fe(II) formation rates were independent of the initial Fe(II) concentrations (Table 2). Fe(II) formation rates were highest with 21.5 µmol Fe(II) g (dry wt peat)$^{-1}$ day^{-1} in December 2005 and decreased over the sampling period to 6.1 µmol Fe(II) g (dry wt peat)$^{-1}$ day^{-1} in October 2006 in zone I. In zone II, Fe(II) formation rates decreased from 4.0 µmol Fe(II) g (dry wt peat)$^{-1}$ day^{-1} to 1.2 µmol Fe(II) g (dry wt peat)$^{-1}$ day^{-1} in November 2006. Only small variations in Fe(II) formation rates could be detected in zone III over the year. Zone IV always showed negligible rates. The maximum amount of Fe(II) formed in peat microcosms ranged from 130 to 180 µmol Fe(II) g (dry wt peat)$^{-1}$ in zone I.

CH_4 formation and pathways of methanogenesis

CH_4 formation was highly spatially variable at the fen site. Only samples obtained from the two sites south of C5 showed a spontaneous formation of CH_4 from 0- to 40-cm depth (Fig. 4). Initial rates of CH_4 formation in samples obtained from these two sites were highest in zone I or II (Fig. 4). CH_3F inhibitor studies demonstrated that aceto-clastic methanogenesis dominated in zones II and III, whereas hydrogenotrophic CH_4 formation was important in zones I and IV (Fig. 4).

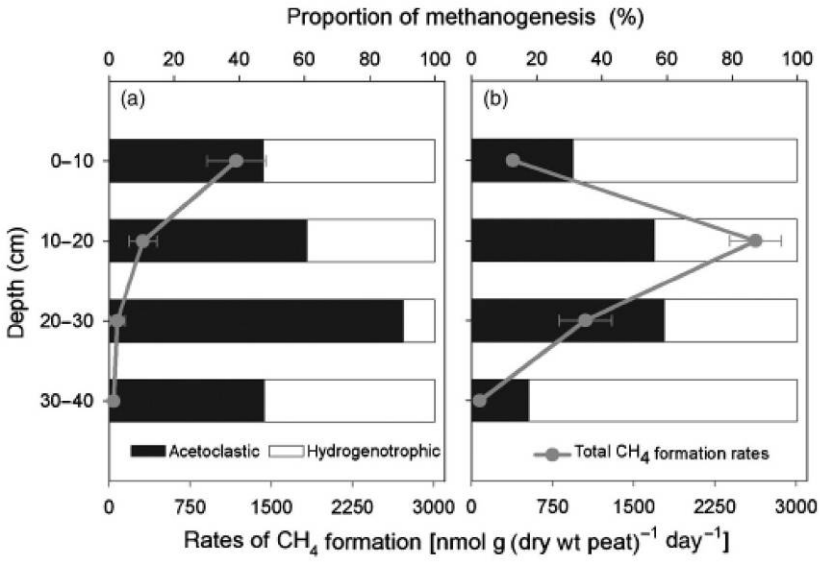

Fig. 4. Depth profile (0–40 cm) of the percentage distribution of the initial acetoclastic and hydrogenotrophic methanogenesis, and initial CH_4 formation rates in peat obtained from two sites (a) and (b) south of C5 in November 2006. Acetoclastic methanogenesis was measured as the difference between control and CH_3F-inhibited samples ($n = 3$). CH_3F, methyl fluoride.

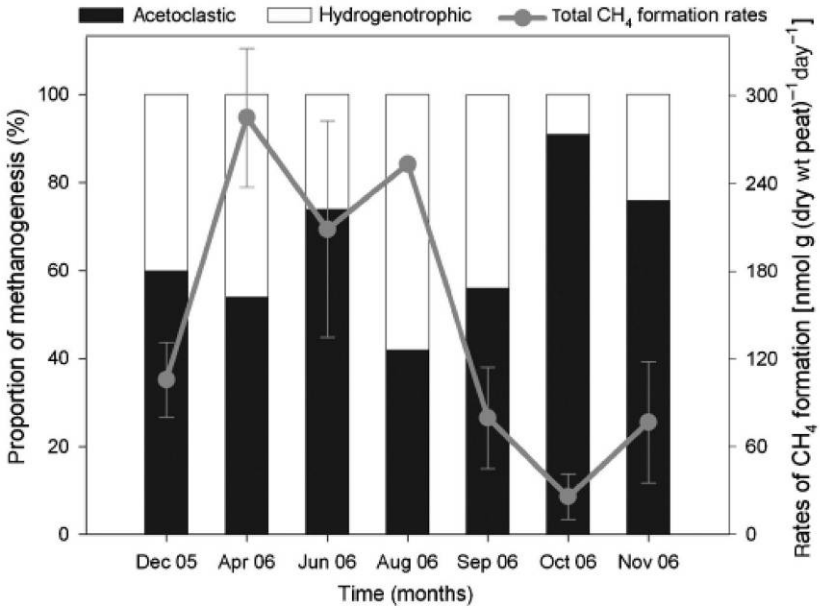

Fig. 5. Seasonal proportion of potential acetoclastic and hydrogenotrophic methanogenesis, and potential CH_4 formation rates in peat obtained from C5 (0–10-cm depth). Deeper zones showed small to negligible CH_4-forming potentials at this site. Acetoclastic methanogenesis was measured as the difference between control and CH_3F-inhibited samples ($n = 3$). CH_3F, methyl fluoride.

In samples obtained from the central part (C5), formation of methane followed Fe(II) formation and started in the most active zone I with an average lag phase of 6 days (data not shown). High rates were determined from April to August with more than 209 nmol CH_4 g (dry wt peat)$^{-1}$ day^{-1} compared with low rates of 106 nmol CH_4 g (dry wt peat)$^{-1}$ day^{-1} in late autumn 2006 (Fig. 5). During the year, acetoclastic methanogenesis dominated the formation of methane in zone I; however, no seasonal trend could be observed. The addition of BES completely inhibited methanogenesis (Fig. 6). Acetate accumulated in peat microcosms of zone I with a rate of 1.9 μmol acetate g (dry wt peat)$^{-1}$ day^{-1} after Fe(II) formation reached a plateau, despite the onset of methanogenesis. Samples obtained from the central part showed no methanogenic activity below 10-cm depth during a prolonged incubation of 31 days. Anaerobic CO_2 formation rates (data not shown) were negligible in these non-CH_4-forming peat samples. However, methanogenesis could be slightly stimulated in peat microcosms by additional H_2 and CO_2. These deeper formerly non-CH_4-forming peat samples consumed H_2 completely, but only $< 3\%$ were recovered in CH_4. In contrast, 18% of the reducing equivalents obtained from H_2 oxidation were recovered in CH_4 in zone I. Deeper peat zones were black, showed a high C/N ratio (Table 1) and were moderately

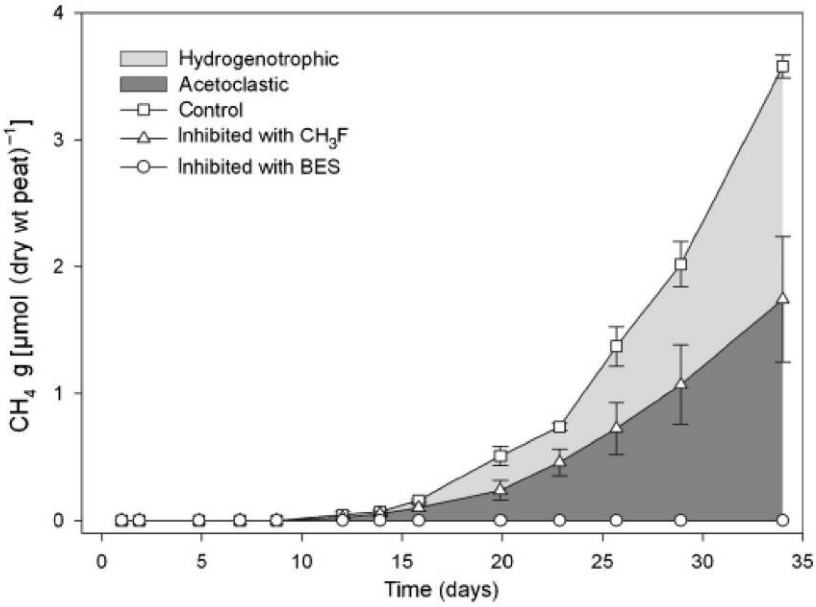

Fig. 6. Total formation of CH_4 and formation of CH_4 in the presence of CH_3F or BES as specific inhibitors in the upper peat zone (0–10 cm) obtained from C5 in August 2006. The proportion of acetoclastic methanogenesis was determined by the difference of CH_4 formation between control and CH_3F-inhibited samples ($n = 3$). CH_3F, methyl fluoride; BES, sodium 2-bromoethanesulfonate.

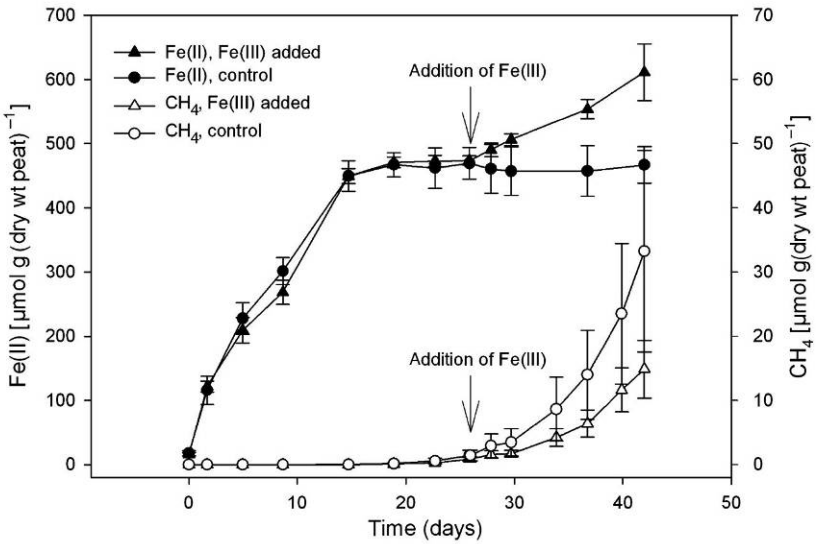

Fig. 7. Effect of additional $Fe(OH)_3$ (13.7 mM) on Fe(II) formation and methanogenesis to peat slurries at the beginning of the CH_4-forming phase after 26 days of incubation ($n = 3$). Peat samples were obtained from C5 in February 2007.

decomposed. In contrast, peat with spontaneous methanogenic activity was brownish and slightly decomposed.

Competing processes in anoxic peat incubations

In most peat microcosms of zone I, Fe(II) formation reached a plateau before CH_4 formation started (Fig. 7, data not shown). Overlapping activities occurred in peat samples obtained in September (data not shown). When peat microcosm suspensions starting to form CH_4 after 26 days of incubation were supplemented with amorphic $Fe(OH)_3$, parallel Fe(II)- and CH_4-forming activities were observed (Fig. 7). Rates of CH_4 formation were inhibited to 54%. The addition of BES to peat microcosm suspensions of zones I and II at the beginning of incubation inhibited the rate of Fe(II) formation to 45% (data not shown).

Discussion

Availability of Fe(III)

The addition of chelating agents and electron shuttling compounds had only small to even inhibitory effects on Fe(II) formation rates and the extent of Fe(II) formation, indicating a good bioavailability of Fe(III) in the fen. Total amounts of Fe(II) formed during anoxic incubations

reached up to 88% of the total Fe(III) content of zone I, suggesting a high microbial available Fe(III) pool at the surface apparently caused by recently precipitated Fe(III) oxides. More crystalline Fe(III) oxide phases likely present in deeper zones of the peat would have decreasing specific surface areas lowering the microbially available surface sites (Roden, 2003), and explain why only 50% of the Fe(III) content could be reduced. In addition, increasing concentrations of Fe(II) in deeper soil solution could decrease the bioavailability of Fe(III) via coating effects on surfaces by Fe(II) (Roden & Urrutia, 1999). Adsorption or precipitation of Fe(II) leads to the formation of an Fe(II) surface phase that limits the extent of Fe(III) oxide reduction (Roden & Zachara, 1996).

The addition of chelators stimulates Fe(III)-reducing activity in pure cultures due to solubilization of the insoluble Fe(III) oxides (Lovley & Woodward, 1996). The redox couple Fe(III) NTA/Fe(II) NTA has a redox potential of +385 mV, which is *c.* 200 mV higher than the corresponding EDTA complex (Straub *et al.*, 2001). Thus, the Fe(III) NTA complex appears to be a more attractive alternative electron acceptor for FeRB. The differences in Fe(II) formation in NTA- or EDTA-amended peat might also be explained by their conformational or sterical differences. NTA occupies three of the available six octahedral positions around Fe(III), whereas EDTA blocks all six octahedral sites surrounding Fe(III) (Haas & Dichristina, 2002). Thus, EDTA can better extract Fe(III) than NTA (Tandy *et al.*, 2004). However, EDTA will also bind Fe(III) more strongly, and microbial cell surfaces have to compete with this strong chelator for Fe(III) in order to reduce it (Haas & Dichristina, 2002). This competition might explain the slightly inhibitory effects of EDTA on the rate and extent of Fe(III) reduction, especially in deeper peat. Recently it was shown that the addition of NTA improves Fe(III) reduction, not by Fe(III) solubilization but by extraction of humic substances from soil (Nealson & Saffarini, 1994; Luu *et al.*, 2003). The solubilized humic materials are able to access and reduce more of the insoluble Fe(III), thus indirectly improving Fe(III) bioavailability. Humic substances derived from peat degradation can also serve as chelators or electron shuttling compounds (Lovley *et al.*, 1998; King & Garey, 1999; Nevin & Lovley, 2002). The high dissolved organic carboncontent of the fen approximated 100 mg L^{-1}. This dissolved humic material might contain enough compounds that can accelerate Fe(III) reduction superseding supplemental electron shuttling compounds like AQDS or supplemental chelators. Because natural dissolved organic matter plays a stimulating role in Fe(III) reduction in terrestrial and aquatic habitats, the microbial reduction of Fe(III) appears to be favored as a terminal electron-accepting process in humic-rich habitats such as peatlands, especially in minerotrophic peatlands that can receive iron from surrounding terrestrial soils.

Metabolic types of microorganisms involved in Fe(III) reduction

Enriched fermentative FeRB were highly abundant [up to 10^7 cells g (fresh wt peat)$^{-1}$] in surface peat and approximated 0.2% of all DAPI counted cells. The amount of reducing equivalents theoretically obtained from glucose oxidation approximated 8–19%, which is even higher than the values reported from other fermenting dissimilatory Fe(III) reducers (Lovley *et al.*, 2004). Because the abundance of fermentors was one or two orders of magnitude higher than the MPN values of acetate- or ethanol-utilizing Fe(III) reducers, fermentors might contribute substantially to the reduction of Fe(III) in this fen. Recently it was shown that the majority of clones derived from Fe(III)-rich coal mining lake sediments are related to *Acidobacteria* (Blöthe *et al.*, 2008). Members of the *Acidobacteria* have been detected in a great variety of ecosystems, including peatlands (Dedysh *et al.*, 2006; Kraigher *et al.*, 2006), and also in this fen (A. Schmalenberger, pers. commun.). Although the physiological capabilities of the vast majority of *Acidobacteria* remain unclear, *Acidobacterium capsulatum* can reduce Fe oxides under anoxic conditions at pH 5 during glucose fermentation (Blöthe *et al.*, 2008), suggesting that *Acidobacteria* are also involved in Fe(III) reduction in fens. The capacity of fermentors to utilize a broad range of electron donors could avoid direct competition with H_2- or acetate-utilizing methanogens.

MPN values of acetate-utilizing Fe(III) reducers cultured at pH 7.0 were significantly lower than those cultured at pH 5.5, indicating that the Fe(III)-reducing population in the fen is well adapted to moderately acidic conditions. The ranking of reducing equivalents recovered in Fe(II), which can be theoretically obtained from supplemental substrate oxidation determined in peat incubations, was formate, H_2 > ethanol > glucose > acetate, suggesting that both formate and H_2 were important substrates for Fe(III) reducers. Ethanol plays a major role in the flow of carbon and reductants in an acidic bog in Finland (Metje & Frenzel, 2005). Plant roots release ethanol as a fermentation product, as a physiological response to hypoxia. A great variety of prokaryotes including *Geobacter* species, but also some sulfate reducers are known to completely oxidize ethanol with Fe(III), whereas *Pelobacter* and *Shewanella* species incompletely oxidize ethanol with Fe(III) (Lovley *et al.*, 2004). In the acidic bog, ethanol appears to be oxidized to acetate either in a syntrophic reaction with methanogenesis as a H_2 sink or as a reductant for Fe(III) (Metje & Frenzel, 2005). Based on the substrate product balances observed in peat microcosms and MPN tubes, ethanol appeared to be both incompletely and completely oxidized with Fe(III). Metabolic profiles of BES-treated MPN tubes demonstrated that the majority of ethanol was completely oxidized to CO_2

and the reductants were mainly transferred to Fe(III). Small amounts of H_2 accumulated in the lowest dilutions, suggesting that the syntrophic partner was inhibited. Without BES, acetate accumulated in equimolar concentrations to ethanol consumption; CH_4 but not H_2 was detected in the lowest dilutions. Because MPN values of ethanol-utilizing Fe(III) reducers without BES were one or two orders of magnitude higher than the values obtained in the presence of BES, incomplete oxidation of ethanol with Fe(III) as sink combined with syntrophic oxidation with methanogens or H_2-utilizing Fe(III) reducers appear to be the main pathways of anaerobic ethanol oxidation in this fen.

Many H_2-utilizing methanogens can transfer electrons to poorly crystalline Fe(III) oxide, both directly and via electron shuttling with extracellular quinones, even when growth and methanogenesis are inhibited (Bond & Lovley, 2002). The contribution of methanogens to Fe(III) reduction is discussed as an alternative explanation for the inhibition of methanogenesis in Fe(III)-rich ecosystems (Bond & Lovley, 2002; van Bodegom *et al.*, 2004). The addition of BES inhibited the rate of Fe(II) formation in peat microcosms to *c.* 45%. Because the compound BES is an analog of the methyl-coenzyme M unique to methanogens (Oremland & Capone, 1988), methanogens appeared to be involved in the reduction of Fe(III). Nevertheless, unspecific side effects of BES could not be ruled out. Overlapping Fe(III)-reducing and CH_4-forming activities were observed in peat microcosm experiments of September 2006. Concomitant Fe(III)-reducing and CH_4-forming activities were observed when amorphous Fe(III)-oxyhydroxides were added to peat microcosms reaching the methanogenic phase (Fig. 7). The rates of CH_4 formation were only inhibited to 54% during ongoing stimulated Fe(II)-forming activities, suggesting (i) the utilization of noncompetitive substrates or (ii) a contribution of methanogens to Fe(III) reduction. When rice paddy soils are amended with ferrihydrite, methanogenesis is rapidly but incompletely inhibited, and acetate concentrations are lowered to values stimulating the activity of *Methanosaeta* (Lueders & Friedrich, 2002). Thus, a shift in the active methanogenic community might have also occurred in peat microcosms.

Competition for electron donors

The abundance of enriched FeRB decreased only slightly with increasing soil depth and cannot explain the negligible Fe(III)-reducing activity in soil below 30 cm. C5 displayed very low microbial respiratory or methanogenic activities in deeper peat layers apparently due to substrate limitation caused by poor peat quality. Spatial heterogeneity of geochemical conditions and microbial activities are often described in peatlands and also for this fen (Paul *et al.*, 2006). When this deep peat material was supplemented with H_2/CO_2, H_2 oxidation was coupled mainly with Fe(III) reduction, and only $< 3\%$ of the reducing equivalents were recovered in CH_4. However, we cannot rule out that H_2 and CO_2 consumption was first coupled to acetogenesis yielding acetate (Drake, 1994), and that acetate was used by Fe(III) reducers, synthrophic acetate oxidizers or acetoclastic methanogens. Acetate accumulated in peat microcosms when Fe(III) reduction reached a plateau, indicating that acetate is an important intermediate during anaerobic peat degradation in this fen.

The small stimulation of Fe(III) reduction by supplemental acetate in deeper peat microcosms also supplemented with $Fe(OH)_3$ might be due to the relatively high initial concentrations of acetate (2 mM) used. At an extracellular pH_{out} of 4.7, undissociated acetic acid can permeate the cellular membrane and lead to a decoupling of the membranous proton motive force (Luli & Strohl, 1990). Methanogens are very sensitive to volatile fatty acids at acidic pH (van den Berg *et al.*, 1976; Wang *et al.*, 1997), and acetate inhibits methanogenesis in acidic peat (Williams & Crawford, 1984; Landsdown *et al.*, 1992; Horn *et al.*, 2003). A rapid *in situ* turnover rate of acetate might avoid toxic effects.

Anoxic incubation with CH_3F showed that the proportion of acetoclastic to total methanogenesis approximated 60%. After heavy rainfalls in October 2006, the acetoclastic CH_4 formation became even more important. However, seasonal changes likely caused by variations in the availability of methanogenic substrates could not be observed. The high fraction of CH_4 produced from acetate is consistent with results obtained from tracer and inhibitory experiments from a mesotrophic fen (Galand *et al.*, 2005), but not with other incubation studies (Williams & Crawford, 1984; Lansdown *et al.*, 1992; Horn *et al.*, 2003) or isotope data analysis (Popp *et al.*, 1999; Chasar *et al.*, 2000) of acidic bogs and northern peatlands. Oligotrophic fens, ombrotrophic bogs and *Sphagnum* sp.-dominated peatlands are often hydrogenotrophic (Galand *et al.*, 2005), whereas acetoclastic methanogenesis dominates in peatlands with higher plant communities, e.g. *Carex* sp. (Kelley *et al.*, 1992; Galand *et al.*, 2005; Rooney-Varga *et al.*, 2007), similar to the fen in the Lehstenbach catchment, which is covered with *Carex*, *Juncus*, *Molinia* and *Eriophorum* species.

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IMPACT OF MANIPULATED DROUGHT AND HEAVY RAINFALL EVENTS ON PEAT MINERALIZATION PROCESSES AND SOURCE-SINK FUNCTIONS OF AN ACIDIC FEN

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Abstract

We manipulated summer drought and heavy rainfalls in a fen field site to estimate changes of peat decomposition and source-sink functions. CO_2 formation rates and exoenzymatic activities increased in the most active surface layer during initial water table drawdown but extreme drying did not further increase these activities. Stimulated activities in deeper oxygenated peat layers did not substantially contribute to CO_2 emissions. No phenol oxidase activity was determined. Rewetting of peat after drying did not lead to a CO_2 flush like in terrestrial soils. Weather extremes yielded a higher availability of nitrate, Fe(III), and sulfate and prolonged the onset of methane formation. Sulfate was exported to a nearby stream. The increase of extreme weather conditions like summer droughts and heavy rainfall events that are predicted for the next decades might not affect carbon storage but strengthen the sink function for nitrate and iron and the source function for sulfate of peatlands.

Keywords: weather extremes, Fe(III) reduction, exoenzymes, porewater biogeochemistry, basal soil respiration

Introduction

In the boreal hemisphere, peatlands maintain high water levels and, consequently, an imbalance between net primary production and decomposition leading to the storage of a large carbon (C) pool [*Gorham*, 1991]. Northern peatlands represent about one third of the global terrestrial C pool [*Gorham*, 1991] and emit approximately 3-7% of the global annual emission of the greenhouse gas methane (CH_4) [*Aselmann and Crutzen*, 1989]. High-latitude regions are expected to experience a temperature increase and a decrease in annual summer precipitation in most European regions as a result of global climate change [*IPCC*, 2007]. A persistent change in the water level can induce changes in the peatland plant community structure leading to an increase in vascular plant cover [*Weltzin, et al.*, 2000; *Weltzin, et al.*, 2003]. Climate models also predict an increase of extreme weather conditions like summer droughts and heavy rainfall events during the next decades [*IPCC*, 2007]. These single events might have more pronounced effects than long-term shifts of the water table in peatlands.

Rates of CH_4 production are correlated with water table depth, pattern and frequency of drought events [*Hughes, et al.*, 1999]. CH_4 emissions are negatively affected by a water table lowering due to a renewal of electron acceptor pools [*Zehnder and Stumm*, 1988; *Nedwell and Watson*, 1995]. Thus, the predicted climatic changes may alter carbon storage and greenhouse gas fluxes, but also the source-sink functions of peatlands regarding nitrogen, iron, sulfur, and alkalinity. Peatlands can act as long-term sinks for deposited protons, nitrate, and sulfate [*Alewell and Giesemann*, 1996; *Küsel and Alewell*, 2004]. Up to 70% of the impacted acidity can be neutralized in forested wetlands by Fe(III)- and sulfate-reduction [*Sahin, et al.*, 1998]. However, it is also shown that minerotrophic conifer swamps retain sulfate during wet years and export it during dry years [*Lazerte*, 1993; *Devito and Hill*, 1999].

Since drying increases the availability of oxygen to aerobic soil decomposers, this may increase the CO_2 flux from peat to atmosphere and drastically affect the C pool of peatlands [*Hogg, et al.*, 1992; *Blodau, et al.*, 2004; *Hirano, et al.*, 2007]. For the mineralization of complex organic matter, especially the hydrolytic exoenzymes; phosphatases, ß-glucosidases, lipases, proteases, and esterases processes are important [*Chròst*, 1991; *Sinsabaugh and Moorhead*, 1994; *Foreman, et al.*, 1998; *Gusewell and Freeman*, 2005; *Rejmankova and Sirova*, 2007]. Biodegradation in peatlands appears to be inhibited due to the absence of oxygen that prevents phenol oxidases from eliminating phenolic compounds [*Freeman, et al.*, 2001]. Oxygen constraints on phenol oxidases can minimize the activity of hydrolytic exoenzymes responsible for peat degradation. Thus, increased peat aeration, as a result of

drought events could eliminate this critical mechanism restricting the re-release of CO_2 to the atmosphere [*Freeman, et al.*, 2001].

Numerous studies were performed to evaluate effects of long-term water table drawdown or rewetting of managed peatlands on changes in vegetation patterns, microbial activities, gas emissions and porewater chemistry [*Pfadenhauer and Klotzli*, 1996; *Baum, et al.*, 2003; *van Dijk, et al.*, 2007; *Waddington and Day*, 2007; *Zak and Gelbrecht*, 2007]. Also short-term laboratory and field studies suggest that drying associated with climate change stimulates microbial CO_2-respiration but minimizes CH_4 emissions from peatlands [*Pulford and Tabatabai*, 1988; *Moore and Dalva*, 1993; *Strack and Waddington*, 2007]. Few studies address exoenzymatic activities that are stimulated in a peatland subjected to field-based experimental water table lowering due to reduced inhibitory effects of iron and phenolic compounds [*Freeman, et al.*, 1996]. However, the effects of single drought followed by heavy rainfall events on peat decomposition, anaerobic microbial processes, and porewater biogeochemistry have been rarely studied in a field experiment. The following study aimed to manipulate extreme weather conditions in a minerotrophic fen field site. We examined basal soil respiration, exoenzymatic activities, and anaerobic microbial processes in combination with detailed porewater analyzes to estimate potential changes of source-sink functions of peatlands due to global change.

Materials and Methods

Site Description and Experimental Setup

Samples were obtained from an acidic fen (Schlöppnerbrunnen, fen area: 0.8 ha) in the Lehstenbach catchment area located in the northern Fichtelgebirge region in east-central Germany at 50°7’54” N, 11°52’51” E at an altitude of 700 m above sea level. Soils are Histosols on granite bedrock. Mean annual precipitation of the Lehstenbach catchment area between 1971-2000 approximated 1163 mm and mean annual air temperature was 5.3°C [*Foken*, 2003]. Mean peat temperature measured in 10 cm depth was 10.2 and 11.9°C for the manipulations 2006 and 2007, respectively.

The Schlöppnerbrunnen fen has an average peat accumulation of about 50 cm and the vegetation is dominated by *Carex canescens*, *Carex rostrata*, *Juncus effuses, Molinia caerulea* and *Eriophorum vaginatum.* Six different plots (7.2 m x 5 m each) equipped with piezometers for groundwater level monitoring down to 40 cm depth were used for water table drawdown and rewetting experiments. Air temperature, precipitation, air humidity, wind

speed and wind direction were measured at 2 m height. The groundwater flows through the peat from the north to the south, and plots showed a water saturation gradient from east to west due to a slight slope in the field site. Four plots showed similar vegetation patterns, in two also *Sphagnum* spp. were found. Thus, two plots with similar vegetation and hydrological conditions were grouped as manipulated (D5) and control (C5) plots.

Due to the very low hydraulic conductivity in the subsoil of less than 10^{-7} m s^{-1}, the groundwater level was lowered by drainage tiles that were installed at 1 m depth perpendicular to the groundwater flow direction upstream and downstream of the experimental plots at a distance of about 1 m in summer 2005. To account for possible artifacts, drainage tiles were installed at the control plots as well. During the 2006 experiment, and during the first weeks of the 2007 experiment, water level in the drainage tiles was lowered down to 1 m below surface by submersed pumps every second or third day only at the manipulated plots. During the second phase of the 2007 manipulation experiments, water level in the drainage tiles at the manipulation sites were continuously kept at 1 m below surface by an automatic pump system. Thus, the manipulated sites were to a high degree disconnected from the lateral groundwater flow. In addition, transparent greenhouses were installed at the first day of pumping on the manipulated plots to exclude rainwater. Since side walls were open to allow free air circulation under the roof, no significant increase of soil temperature was measured in the uppermost soil layer. The temperature ranged between 9-14°C at 10 cm depth during the manipulation periods.

Water table drawdown was induced at the manipulation plots for 6 weeks from 14th August to 27th September in summer 2006 and for 9 weeks from 10th May to 19th July in 2007. At the end of the water table drawdown experiments, the manipulated sites were rewetted by irrigation of artificial rainwater. In 2006, 11.9 m^3 were irrigated within 10 hours which corresponds to 110 mm related to the irrigated area of 108 m^2. In 2007, 12 m^3 were irrigated on 19 July 2007, and another 7.7 m^3 on 23 July 2007, corresponding to 182 mm rainfall. Irrigation intensity was 10 mm h^{-1} and 11 mm h^{-1} in 2006 and 2007, respectively. Artificial rainwater was prepared by adding minor amounts of NH_4NO_3, Na_2SO_4 and K_2SO_4 to distilled water corresponding to mean concentration in open field precipitation.

Peat Core and Soil Water Sampling

Peat and soil water solution were sampled on the manipulated D5 plot and the corresponding control plot C5 in 2006 at the beginning and end of water table drawdown (after 42 days), and at 1, 7, and 19 days after rewetting. During the manipulation experiment of 2007 samples

were obtained at the beginning, during the period of water table drawdown (after 21 and 35 days) and at the end of water table drawdown (after 65 days), and 6 and 20 days after rewetting. Three replicate peat samples from 0-40 cm depth (Table 1) were obtained using peat corers with a diameter of 8 cm for microcosm incubation studies and with a diameter of 2 cm for measurement of exoenzymatic activities. Fresh plant litter was removed from the top, cores were separated in 5 or 10 cm depth segments, and replicate peat zones were pooled, respectively. Peat samples were transported to the laboratory in airtight plastic bags or tubes at 4°C. Samples were processed within the same or the next day.

Soil solution was obtained close to the 8 cm core sampling sites with 10 mL disposable syringes (Terumo, Belgium) from prior installed Rhizon samplers (Eijkelkamp, Netherlands) in 1, 5, 10, 15, 20, 25, 30, 35, and 40 cm depth. Soil water was analyzed for Fe(II), Fe(III), NO_3^-, NH_3^+, SO_4^{3-}, PO_4^{3-}, and pH in 2006 and 2007, and additionally for the concentration of polyphenolic compounds in 2007.

To estimate the presence of oxygen in the peat cable funnels (700 x 9 x 8 mm) containing approximately 13 mM particulate black FeS embedded in a 2% agar matrix (FeS-redox-probes) were used [*Reiche, et al.*, 2008]. FeS-redox-probes were equilibrated for a minimum of 7 days in the field. Thus, the maximum penetration depth of oxygen during the elapsed incubation period is estimated. Color change from black to brow indicated the presence of oxygen due to the oxidation of FeS to Fe(III)- oxyhydroxides. Transition of colors occurred mostly within a few mm.

Peat Microcosm Studies

To study basal soil respiration rates and anaerobic CO_2 formation, 20 g (fresh wt peat) were placed into sterile 180 mL incubation flasks (Mueller & Krempel, Buelach, Switzerland) in three replicates either under sterile air or under a continuous flow of sterile argon, respectively. Flasks were closed with rubber stoppers and screw-caps, and were incubated in the dark at 15°C with an initial overpressure of approximated 100 mbar. Headspace concentrations of CO_2 were measured at 4 time intervals during an incubation of 24 h.

Fe(III) reduction and formation of CH_4 were determined at selective time intervals during an incubation period of 31 days at 15°C in triplicate microcosms as previously described [*Reiche, et al.*, 2008]. Fe(III) reduction rates were determined from the linear increase of Fe(II) formation determined after acid extraction. To differentiate between hydrogenotrophic and acetoclastic methanogenesis methyl fluoride (CH_3F, final headspace concentration of 1%

in 2006 and of 1.4% in 2007) was added as selective inhibitor [*Frenzel and Bosse*, 1996] to three replicate microcosms as previously described [*Reiche, et al.*, 2008].

Table 1. Geochemical characteristics of the control plot C5 and the manipulation plot D5 over depth (0-40 cm) (n=2).

Depth	pH[a]	P_{total}	Fe_{total}[b]	Al_{total}	LOI[c]	N_{total}	C_{total}	H_{total}	S_{total}	C/N
cm (Plot)		µmol g (Dry wt Peat)$^{-1}$					%			
0-10 (C5)[d]	4.7	58	211	7.2	70	2.0	36.2	4.5	0.3	18.6
10-20 (C5)[d]	4.7	48	112	4.1	83	2.1	47.3	5.4	0.3	22.6
20-30 (C5)[d]	4.6	26	138	3.3	88	1.5	51.5	5.5	0.2	35.3
30-40 (C5)[d]	4.8	32	88	2.3	87	1.2	50.4	6.0	0.2	40.6
0-10 (D5)	4.7	48	253	43.8	75	1.7	36.4	4.5	0.3	21.8
10-20 (D5)	4.8	39	133	6.7	62	1.3	37.6	4.4	0.2	29.1
20-30 (D5)	4.7	26	122	4.3	92	1.3	55.3	6.7	0.3	43.6
30-40 (D5)	4.7	32	133	3.4	85	1.3	51.0	6.0	0.3	38.2

[a] Soil Water pH (n=10)

[b] n=12

[c] Loss on Ignition

[d] Some data were obtained from Reiche et al. [2008]

Exoenzymatic Activities

Activities of phosphatases, ß-glucosidases, lipases, esterases and proteases, and phenol oxidases were determined using methylumbelliferyl phosphate (MUF-P, 30 mM), MUF-ß-gluco-pyranoside (MUF-ß-Glc, 10 mM in 50% ethylene glycol monomethyl ether), diacetylfluorescein (FDA, 2.4 mM in 98% acetone), and L-3-3,4-dihydroxyphenyl-alanine (L-dopa, 10 mM) as model substrates, respectively [*Schnurer and Rosswall*, 1982; *Hoppe*, 1993; *Pind, et al.*, 1994]. Peat slurries from every depth zone and sampling site were prepared in a beaker by mixing deionized water and field fresh peat (25 mg [fresh wt peat] mL^{-1}). Due to stirring of peat slurries, the amount of dissolved phenolics increased by approximated 3 mg phenolic compounds L^{-1} (e.g. at C5, 0-10 cm) compared with the corresponding porewater data. Four mL of the peat suspensions were filled into glass tubes in triplicates and stock solutions of the model substrates were added to reach a final concentration of 1.5 mM, 1 mM, and 57 µM for MUF-P, MUF-ß-Glc, and FDA, respectively. Phenol oxidases activities

were determined by incubating peat suspensions with a final L-dopa concentration of 5 mM. 200 mg of smashed potato, banana, or apple, known to contain phenol oxidases were added as positive controls. Heat sterilized peat suspensions that were boiled for 5 min before substrate addition were used as control to determine non-enzymatic hydrolyses of the substrates.

The inhibition effect of humic acids on exoenzymatic activities was determined after addition of a commercial humic acid (Fluka, Switzerland) in the range from 0-500 mg L^{-1} to peat suspensions of C5 (0-10 cm). To determine the effect of temperature on exoenzymatic activities, peat suspensions were incubated at 4, 14, 26, 35, 45, 55, 65, and 75°C in drying ovens and water baths; to determine the effect of pH, peat suspensions were adjusted to pH 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 with HCl or NaOH, respectively. The temperature optimum for phosphatases exhibited 45°C and highest activity for FDA-hydrolyzing enzymes (FHE) ranged between 45°-65°C. ß-Glucosidases activity increased until 45°C and was stable up to 75°C. Phosphatases were most active between pH 3-9 with no apparent optimum, whereas ß-glucosidase showed only high activities between pH 3-7. FHE activities were high between pH 4-7 with an optimum at pH 5.

Final substrate concentrations were determined with saturation kinetics (ranging from 0-220 µM for FDA, from 0-6000 µM for MUF-P and from 0-2000 µM for MUF-ß-Glc) and time of incubation was determined by time courses (ranging from 0-120 min). Substrate incubations lasted for 1h (MUF substrates), 30 min (FDA), or 5 min (L-dopa) and were performed in the dark on an orbital shaker (SM, Edmund Bühler, Hechingen, Germany) and than stopped by centrifugation (1600 g, 5 min; tabletop centrifuge T62.1, VEB MLW, Engelsdorf, Germany). Oxic incubation conditions did not affect the extent of hydrolyses. Supernatants of the MUF incubations were diluted 1:20 with deionized water and 11% glycine buffer (1.2 mM glycine adjusted with 10 M NaOH to pH 10), and measured with a fluorescence-spectrophotometer (Perkin Elmer, LS 50 B; em 450 nm, ex 365 nm, slit 2.5). FDA supernatants were mixed 1:1 with 60 mM phosphate buffer (50 mM K_2HPO_4 and 10 mM KH_2PO_4, pH 7.6). Solutions were measured at 490 nm with a photospectrometer (Uvikon 931, Kontron Instruments, Italy). Supernatants of L-dopa incubations were directly measured at 460 nm. Concentrations were calculated using calibrations curves of fluorescein (ranging from 0-30 µM) or 4-methylumbelliferone (ranging from 0-150 µM) obtained in peat slurries. Turbidity and quenching effects by addition of humic acids were corrected using calibration curves with corresponding humic acid concentrations.

Analytical Techniques

Field fresh duplicate peat samples were dried at 105°C for 24 hours to determine dry weights. Headspace gases were measured with Hewlett Packard Co. 5980 series II gas chromatographs according to *Küsel and Drake* [1995]. Gas pressures in flasks were measured with a TensioCheck TC 1066 (Tensio-Technik, Geisenheim, Germany) needle manometer. SO_4^{2-}, PO_4^{3-}, and NO_3^- were analyzed by ion chromatography [*Reiche, et al.*, 2008]. Ammonia was measured spectrophotometrically (Uvikon 931, Kontron Instruments, Italy) by the hypochlorite- nitroprusside method [*Harwood and Huyser*, 1970]. Concentrations of polyphenols were determined following the Folin-Ciocalteu procedure [*Fenner, et al.*, 2005].

C, H, N, S, P_{total} and Fe_{total} of dried (60°C for 48 hours) and milled (Mixer Mill MM301, Retsch, Germany) soil samples were directly analyzed with an elemental analyzer (vario EL, Elementar) or after acid digestion by the molybdenum blue method (Varian, Cary 1E) or after reverse aqua regia decomposition by flame atomic absorption spectrometry (Perkin Elmer, 3300) [*Zak and Gelbrecht*, 2007]. HCl (0.5 M) extractable Fe(II) was measured after centrifugation spectrophotometrically (Uvikon 931, Kontron Instruments, Italy) following the phenanthroline method after *Tamura et al.* [1974]. HCl extractable Fe(III) was calculated after addition of ascorbic acid (0.6% final concentration) from the increase in concentration. HCl extracts amorphous Fe(III) oxides and reduced Fe, including FeS, and $FeCO_3$ [*Heron, et al.*, 1994; *Kostka and Luther*, 1994; *van Bodegom, et al.*, 2003]. Soil water pH was measured by WTW pH Meter (pH 330, Weilheim, Germany) combined with an InLab 423 combination pH micro electrode (Mettler Toledo, Giessen, Germany).

Statistical Analyses

One-way or repeated measures analysis of variance (ANOVA) with the Tukey post-hoc test for multiple comparisons was used to test for significant differences between the control and manipulation plot during the drying and rewetting experiments. Homogeneity was tested using Levene's Test (SPPS 15.0, SPPS Inc., Chicago, Illinois, USA).

Results

Water Table Manipulations and Soil Solution Biogeochemistry

The summer of 2006 was characterized by low precipitation and a high air temperature compared with mean values of 1995-2005 of that region (Table 2). The groundwater water level prior to the start of the manipulation experiment in August 2006 was low and varied

between 28 to 40 cm at D5 and 14 to 32 cm at C5 (Figure 1). Mean oxygen penetration depth approximated 30 ± 1 and 28 ± 7 cm at D5 and C5, respectively. Pumping lowered the groundwater level at D5 below the piezometer depth of 40 cm after 2 weeks of pumping (Figure 1). Groundwater level at C5 also decreased to a maximum of 35 cm. Peat moisture decreased significantly in D5 peat layers of 0-10 cm, 10-20 cm, and 20-30 cm depth from 87 to 82, 65 to 60, and 77 to 72 %, respectively, but not in deeper peat layers of D5. Peat moisture did not decrease in all peat layers of C5. Mean oxygen penetration depth reached 35 ± 9 and 34 ± 2 at D5 and C5, respectively, at the end of pumping. Irrigation with artificial rainwater of 110 mm on September 29 raised the groundwater level at D5 above that of C5. Due to a heavy rainstorm five days later, the groundwater level increased both at D5 and C5 by another 20 cm. Nineteen days after rewetting, mean oxygen penetration depth reached 5 ± 3 and 11 ± 2 at D5 and C5, respectively.

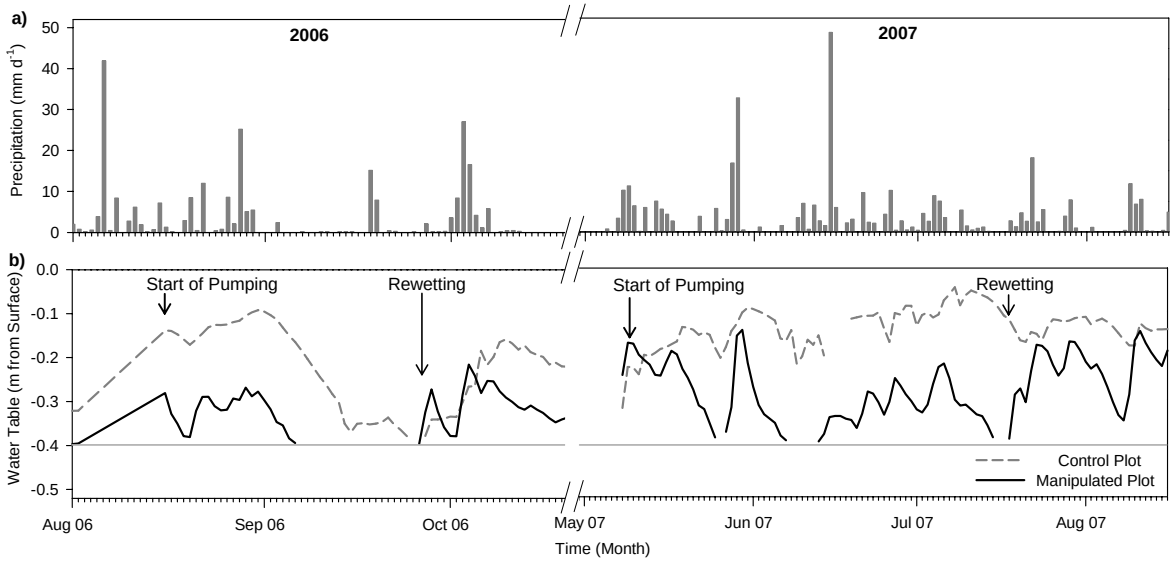

Figure 1. Precipitation (a) and water table fluctuations (b) during water table manipulations at the Schlöppnerbrunnen fen in 2006 and 2007. Water table drawdown at the manipulated plot D5 was induced by roofing and active pumping down of the drainage tiles. Arrows highlight the start of water table manipulations and rewetting as indicated. The grey line (bottom) highlights the installation depth of the piezometers.

Nitrate and sulfate concentrations increased during pumping at D5 from depth averaged concentrations of <1 µM and 300 µM, respectively, to more than 65 µM and 590 µM in the 5-10 cm zone (Figure 2), whereas maximum nitrate and sulfate concentrations in C5 reached 27 µM and 175 µM, respectively. Concentrations of ammonium were negligible both in D5 and C5. Solved Fe(II) increased with increasing soil depth at both plots

and reached up to 130 µM at D5 (Figure 2a) but always less than 80 µM at C5 (data not shown). Pumping resulted in the disappearance of solved Fe(II) concentrations in the upper 20 cm at D5 (Figure 2b). Nineteen days after rewetting nitrate was absent, Fe(II) was again present near the surface, and sulfate reached 80 µM (Figure 2c).

Table 2. Compilation of weather parameter of the water table drawdown from 14 August to 27 September 2006 (42 days) and from 10 May to 19 July 2007 (70 days) compared to the preceding 11 years of the corresponding season.

Year(s)	Air Temperature	Air Humidity	Daily Precipitation	Daily Global Radiation
	°C	%	mm day^{-1}	W m^{-2}
Mean 1995-2005	12.3	80.6	3.0	139.5
Season 2006	13.7	79.0	2.4	122.8
Ratio 2006 / Mean 1996-2005 (%)	111	98	80	88
Mean 1996-2006	13.2	74.9	3.3	196.0
Season 2007	14.4	75.3	4.9	176.5
Ratio 2007 / Mean 1996-2006 (%)	109	101	148	90

The summer of 2007 was characterized by rather low mean daily global radiation and high amounts of precipitation with about 148% of the 1996-2006 mean in that region (Table 2). In contrast, air temperature and air humidity were close to the long-term averages (Table 2). Due to the unusual high precipitation, the groundwater level was very close to the surface which rarely occurred during the growing season of the preceding years. Consequently, rapid near-surface groundwater flow refilled the drainage tiles within a few hours after evacuation during the initial drying phase, but continuous draining decreased significantly the water table in D5 compared to C5 (Figure 1). A stable water table drawdown could be achieved after 35 days after the start of pumping at D5 for a period of 4 weeks. Mean oxygen penetration depth was 3 ± 2 and 6 ± 3 cm at D5 and C5, respectively, prior to the manipulation. During pumping, oxygen penetration depth reached a maximum of 12 ± 4 cm at D5. However, peat moisture content was not significantly affected by pumping. Rewetting with 182 mm artificial rainwater raised the water table at the manipulated sites to levels at 20 cm below surface. Twenty days after rewetting, mean oxygen penetration depth reached 8 ± 3 and 10 ± 3 at D5 and C5, respectively.

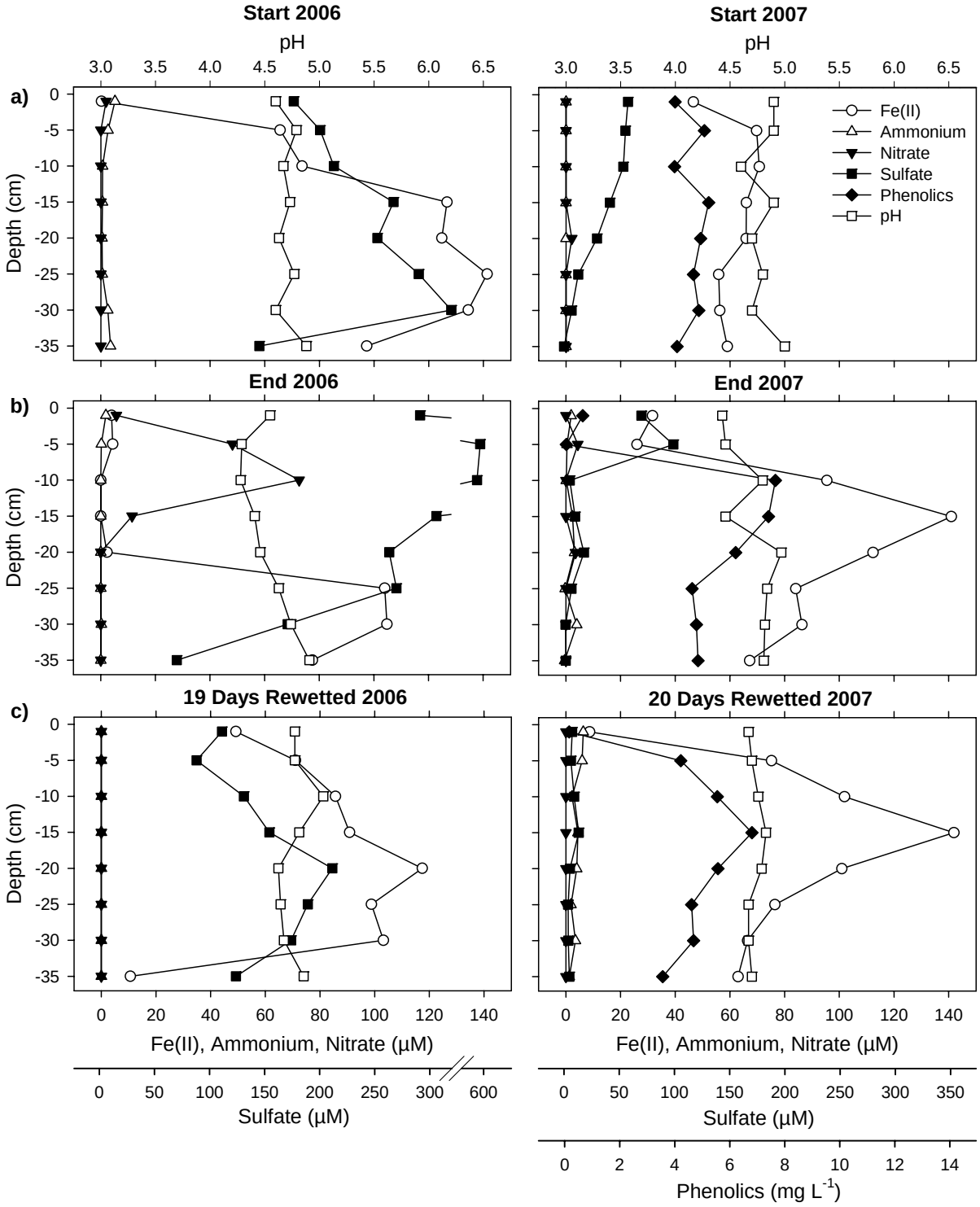

Figure 2. Fe(II), ammonium, nitrate, sulfate, pH, and phenolics concentrations over 35 cm depth obtained with Rhizon samplers during a water table manipulation experiment in 2006 (left) and 2007 (right). Samples were obtained at the beginning (a) of pumping, at the end (b) of pumping, and 19/20 days after rewetting (c) at the manipulated plot D5. No data for phenolic compounds are available for 2006.

In contrast to 2006, concentrations of nitrate and sulfate reached only 5 and 120 µM in the upper 5 cm at the end of the pumping at D5. Sulfate concentrations were negligible at C5. Solved Fe(II) was low near the surface and peaked sharply in 15 cm depth at D5 at the end of pumping. Twenty days after rewetting, high Fe(II) concentrations were also present in 5 cm depth. Concentration of phenolic compounds approximated 4 mg L^{-1} over depth at C5 and D5. At the end of pumping phenolic compounds were absent near the surface, but reached 7.8 mg L^{-1} between 10 and 20 cm depth (Figure 2). Solved phosphate was always below the detection limit (<1 µM) during both manipulation experiments in 2006 and 2007 at all sites. The pH values approximated 4.7 ± 0.3 and 4.6 ± 0.3 at D5 and C5, respectively. The pH decreased to a minimum of 4.3 at the end of pumping in 2006 (Figure 2).

Mineralization Processes

Basal soil respiration, anaerobic CO_2 formation, and exoenzymatic activities were most active in the upper 0-10 cm both at C5 and D5 and decreased strongly with depth (Figure 3, Figure 4). Basal soil respiration rates were up to 1.4-times higher than anaerobic CO_2 formation rates in 0-10 cm depth, but no significant differences were determined in deeper peat layers (Figure 3). Pumping in 2006 did not enhance microbial respiration in D5 in 0-10 and 10-20 cm depth but slightly in 20-30 and 30-40 cm depth. Rates determined after rewetting were lower in 0-10 cm depth, but were slightly higher in 10-20 cm depth in D5. Deeper peat layers were not affected. Pumping in 2007 enhanced basal soil respiration rates in D5 in 0-10 cm depth and after 6 days of rewetting. Rates reached 45.1 µmol CO_2 g (dry wt peat)$^{-1}$ d^{-1} compared to initial activities of 12.2 µmol CO_2 g (dry wt peat)$^{-1}$ d^{-1} (Figure 3). Twenty days after rewetting, basal soil respiration declined to the initial rates of 2007. Basal soil respiration rates at C5 showed a similar trend but reached only 35.8 µmol CO_2 g (dry wt peat)$^{-1}$ d^{-1} in 0-10 cm depth (Figure 3).

The upper peat layers showed the highest but also variable exoenzymatic activities (phosphatases, ß-glucosidases, FDA-hydrolyzing enzymes) over time (Figure 4). In general, activities of phosphatases were always up to 4 times higher compared to ß-glucosidases and FDA-hydrolyzing enzymes. Pumping in 2006 yielded no increase in activities in all soil depths, but phosphatases and ß-glucosidases increased after rewetting in 10-20 cm depth in D5 but not in C5. Water table drawdown in 2007 increased phosphatases, ß-glucosidases, and FDA-hydrolyzing activities (up to 5 times) in surface peat layers of D5, but activities decreased 6 days after rewetting.

No activity of phenol oxidases could be detected during both seasons at both sites in all depths at any time. Peat samples incubated with L-dopa showed higher extinctions than sterilized blanks, and values were in the range of other published data [*Freeman, et al.*, 1996; *Bragazza, et al.*, 2006]. However, a change of the product formation (2-carboxy-2,3-dihydroxyindole-5,6 quinone) over time (0.5-30 min) indicative for a kinetic reaction of the phenol oxidases [*Pind, et al.*, 1994] was not detected with peat samples in contrast to the positive controls used. Since concentrations of phenolic compounds increased during water table drawdown in D5, we tested if the presence of additional humic acids affected the exoenzymatic activities. Activity of ß-glucosidases in peat slurries was decreased by 25% in the presence of additional 50 mg humic acid L^{-1} (which equals an increase from 6.7 to 8.2 mg phenolics L^{-1} in total) and by 37% in the presence of additional 500 mg humic acid L^{-1} (which equals an increase from 6.7 to 27 mg phenolics L^{-1} in total). Activities of phosphatases decreased by 20 % in the presence of 100 to 500 mg humic acid L^{-1}. FDA-hydrolyzing enzymes activities were not affected by the presence of additional humic acids.

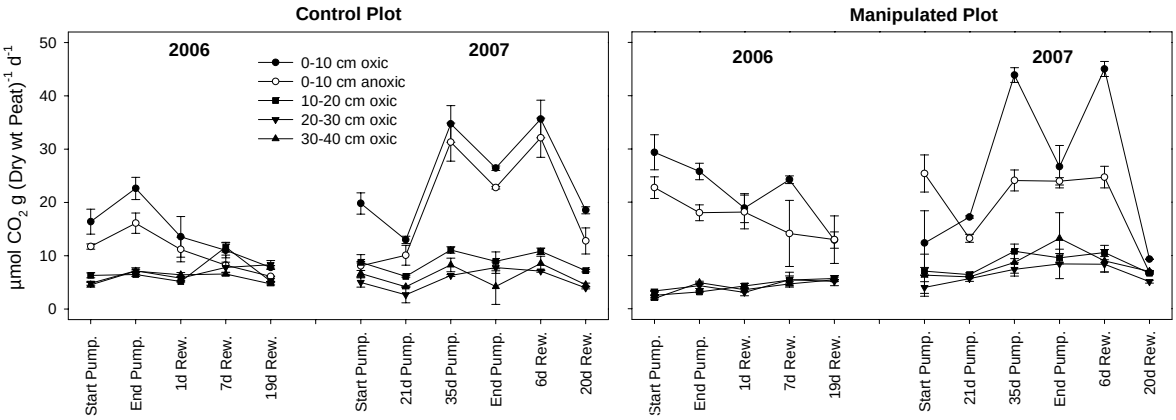

Figure 3. Basal soil respiration and anaerobic CO_2-formation rates (n=3) in oxic (0-40 cm) and anoxic (0-10 cm) microcosm experiments of the control plot C5 (left) and the manipulated plot D5 (right) of the Schlöppnerbrunnen fen in 2006 and 2007. Anoxic peat incubations below 10 cm showed no difference in accordance to oxic incubations. D: Day(s), Pump.: Pumping, Rew.: Rewetted

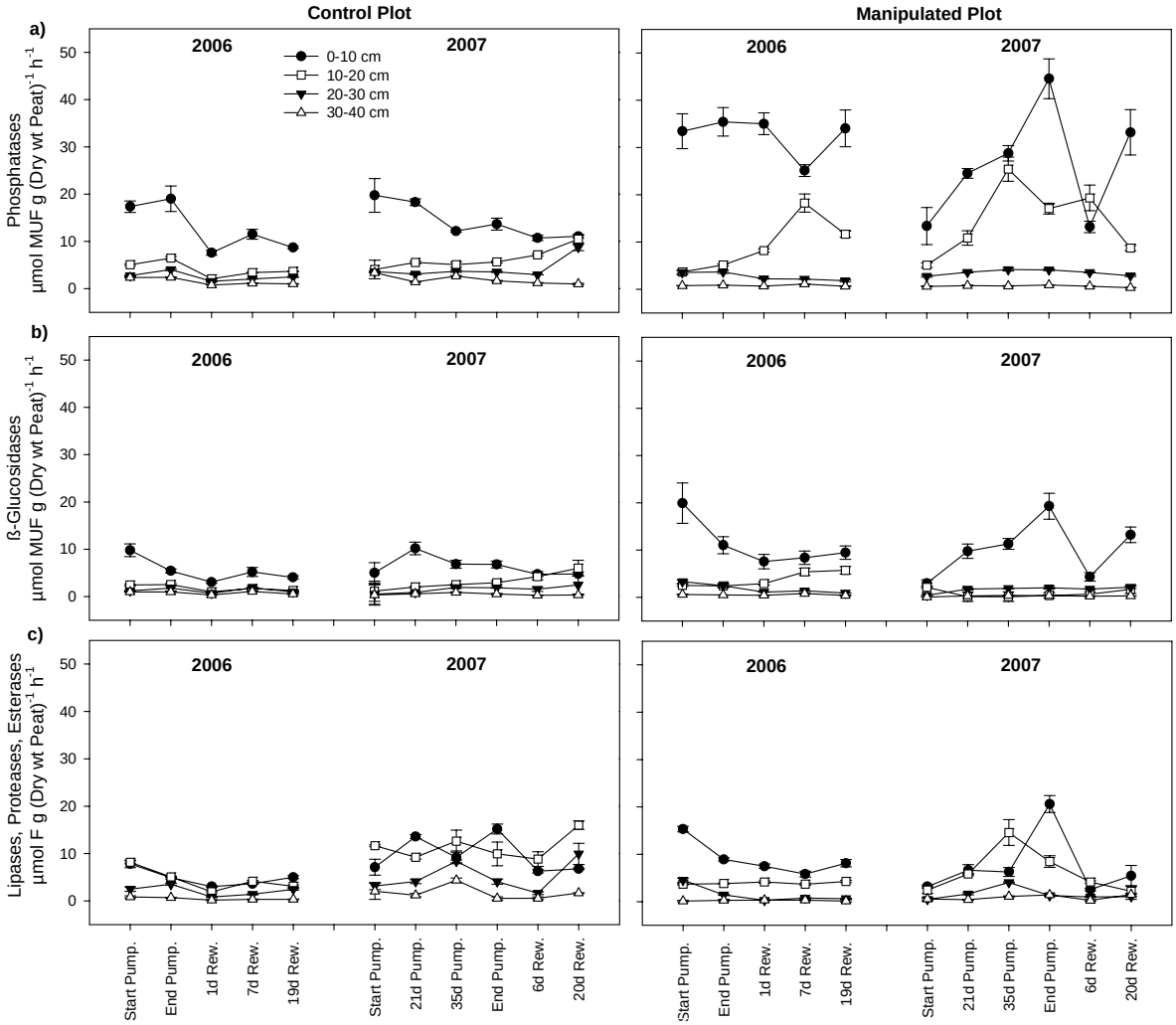

Figure 4. Potential exoenzymatic activities (n=3) of phosphatases (a), ß-glucosidases (b) and FDA-hydrolyzing enzymes (c) in peat obtained from 0-40 cm depth from the control plot C5 (left) and the manipulated plot D5 (right) of the Schlöppnerbrunnen fen in 2006 and 2007. D: Day(s), Pump.: Pumping, Rew.: Rewetted

Formation of Fe(II) and CH_4

The upper peat layer showed the highest Fe(II) formations rates at both sites. Fe(II) formation rates and final Fe(II) concentrations formed at the end of incubation when Fe(II) formation reached a plateau were always higher at D5 compared with C5 apparently due to spatial heterogeneities of the Fe_{total} content in the solid phase (Table 1). Fe(II) formation rates of peat soil obtained 2007 from 0-10 cm depth increased during water table drawdown in D5 and reached similar values compared to Fe(II) formation rates of peat obtained 2006 from 0-10 cm depth at start and end of pumping (Figure 5). Fe(II) formation rates declined again after rewetting 2007 to those obtained at the beginning of the manipulations. Rates of Fe(II) formation and final Fe(II) concentrations showed a positive correlation ($r^2 > 0.8$). Both the

Fe(II) concentrations measured at the beginning of the anoxic incubation (data not shown) and the final Fe(II) concentrations measured at the end of incubation increased in soil obtained 2006 after rewetting down to 30-40 cm depth (Figure 5). In contrast, rewetting in 2007 yielded only increased initial Fe(II) concentrations in 0-10 cm depth. Final Fe(II) concentrations for C5 were similar for 2006 and 2007.

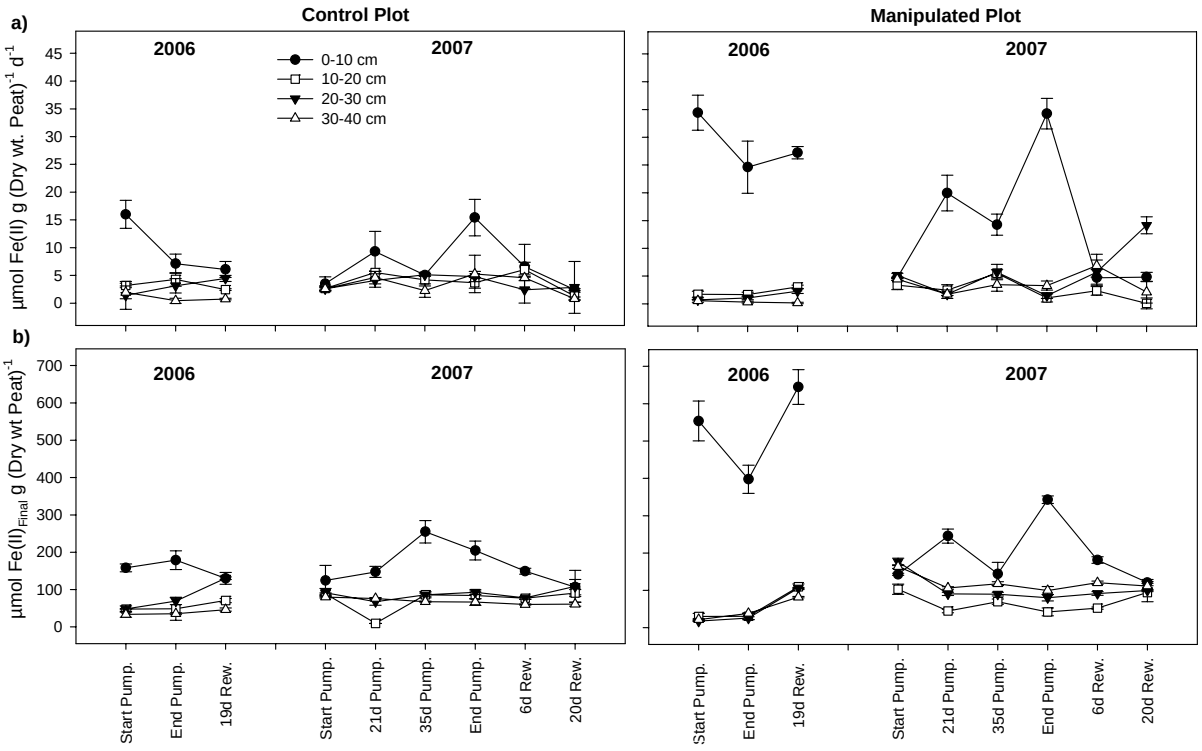

Figure 5. Initial Fe(II) formation rates (a) and final Fe(II) concentrations when Fe(II)formation reached the plateau (b) (n=3) in anoxic microcosm experiments with peat obtained from 0-40 cm depth of the control plot C5 (left) and the manipulated plot D5 (right) of the Schlöppnerbrunnen fen in 2006 and 2007. D: Day(s), Pump.: Pumping, Rew.: Rewetted

In 2006 and 2007, methanogenesis started with a delay of approximately 11 days in anoxic peat incubations of the upper peat zone (0-10 cm). The onset of CH_4 formation was delayed to 21 days 19 days after rewetting. In most peat incubations CH_4 formation started when the Fe(II) concentration plateau was reached, but overlapping activities were observed at the end of drying in 2006 and at the beginning of the water table drawdown in 2007. CH_4 formation rates decreased from 2450 nmol CH_4 g (dry wt peat)$^{-1}$ d^{-1} at the end of pumping 2006 to 225 nmol CH_4 g (dry wt peat)$^{-1}$ d^{-1} 19 days after rewetting at D5. Methanogenesis increased from lowest rates during water table drawdown in mid June with 55.7 nmol CH_4 g (dry wt peat)$^{-1}$ d^{-1} up to 560 nmol CH_4 g (dry wt peat)$^{-1}$ d^{-1} after rewetting at D5 (Figure 6). Thus, no clear impact of water table manipulations could be determined. CH_3F inhibitor

studies demonstrated that acetoclastic methanogenesis dominated the formation of CH_4 in zone I and yielded typically 50% to 70% (Figure 6). Nevertheless, no shift of methanogenic pathways could be observed during the water table manipulations.

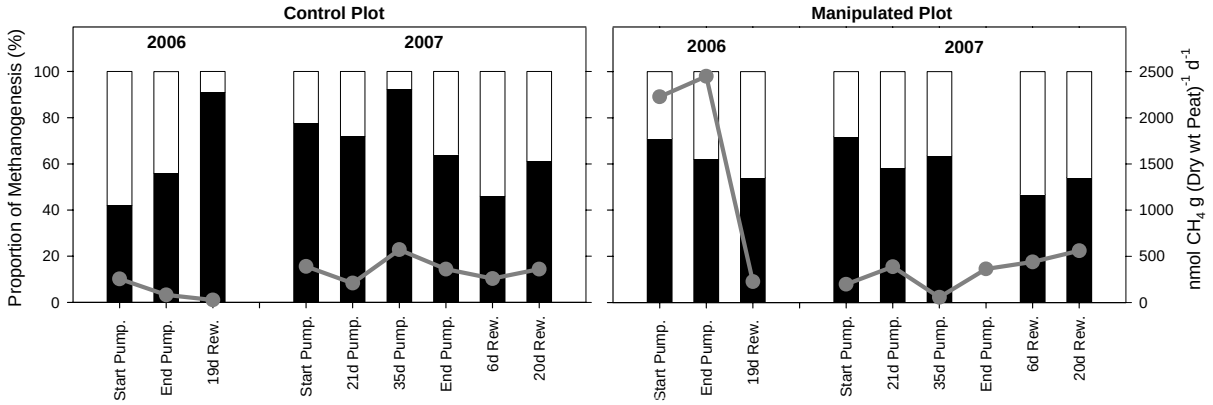

Figure 6. Proportion of potential acetoclastic (black bars) and hydrogenotrophic (white bars) methanogenesis, and potential CH_4 formation rates (lines) in peat obtained from the control plot C5 (left) and the manipulated plot D5 (right) of the Schlöppnerbrunnen fen in 0-10 cm depth during 2006 and 2007. Acetoclastic methanogenesis was measured as the difference between control and CH_3F-inhibit samples (n=3). D: Day(s), Pump.: Pumping, Rew.: Rewetted

Discussion

Increased extreme weather conditions

Climatic warming together with decreasing annual rainfall will lower the water levels in boreal fens by 14–22 cm [*Roulet, et al.*, 1992]. Vascular plant cover might increase in northern wetlands in response to global warming [*Weltzin, et al.*, 2000; *Weltzin, et al.*, 2003; *Walker, et al.*, 2006] and shift methanogenic pathways toward increased acetotrophy and CH_4 formation [*Hines, et al.*, 2008]. Thus, this fen site dominated by vascular plants and acetoclastic methanogenesis might be a good model to study the impact of increasing extreme weather conditions like summer droughts and heavy rainfalls on peat decomposition processes in peatlands. The summer 2006 was characterized in that region by a prolonged dry period starting in June followed by a heavy rainfall, and we intensified these extremes by further lowering the groundwater level below the piezometer depth of 40 cm at the manipulated sites of the fen from the middle of August until September. Peat moisture decreased significantly in D5 but not in C5. The sprinkler irrigation at D5 was amplified by a

natural heavy rainfall 5 days later, and the groundwater level increased to 20 cm below surface.

To study also the initial effects of groundwater lowering, we started 2007 the manipulation already in May and elongated the drying period. As a result of the preceding cold and humid weather period before, the oxygenated peat zone was shallow due to the high water level. Despite the fact that the precipitation sum of 343 mm during the 10 weeks of the manipulation 2007 amounted to 29% of the long-term annual mean, pumping yielded a decrease of the groundwater table at D5 by 20 cm lower than C5, and mean oxygen penetration depth increased from 3 ± 2 cm to a maximum of 12 ± 4 cm at D5. The comparison of the oxidized zones of the FeS-redox-probes and the piezometer level data demonstrated that even under non water-saturated conditions in the peat surface layers, the oxygen availability was not always sufficient for a chemical oxidation of Fe(II). However, the FeS-redox probes record the maximum oxygen penetration depth during the exposure period [*Reiche, et al.*, 2008]. Thus, the actual groundwater level measured at the time of the FeS-redox probe removal is not indicative under fluctuating groundwater level conditions. Although several probes were installed at each plot to improve the significance of oxygen penetration depth, small scale heterogeneities due to the local oxygen leakage from plant roots [*Kostka and Luther*, 1995; *Roden and Wetzel*, 1996; *Frenzel, et al.*, 1999] cannot be recorded.

Enhanced peat decomposition during water table drawdown

Lowering of the water level can drastically affect the C pool of peatlands. Soil respiration and microbial biomass carbon increase, whereas photosynthesis, CH_4 production, and CH_4 emission decrease in peatland mesocosms exposed to a lowered water table [*Blodau, et al.*, 2004]. Aerobic soil respiration rates are up to 4.3 times higher than anaerobic respiration rates [*Bridgham and Richardson*, 1992; *Moore and Dalva*, 1997; *Bergman, et al.*, 1999], and the C-emission rate is three-fold higher at dry peatland locations compared with wet peatlands indicating enhanced decomposition [*Jaatinen, et al.*, 2008]. During the water table drawdown of 2007, basal soil respiration rates of the surface peat layer (0-10 cm) increased by a factor of 2.1 (±0.4), but basal soil respiration rates were only 1.4 (±0.5) times higher than anaerobic CO_2-formation rates (Figure 3). Aerobic and anaerobic CO_2-formation in deeper peat layers were not affected by the lowered water table in 2007. When the low initial water table was further lowered during pumping of 2006, CO_2 formation rates of the surface layer leveled off, and only rates of deep peat layers (20-30 and 30-40 cm depth) increased.

However, rates of deeper peat layers were low due to the poor peat quality and high C/N ratio (Table 1). The drought experiment 2006 did also not have a significant effect on CO_2 field emissions (Muhr, J., manuscript in preparation, 2008). Thus, extreme droughts of 2006 did not further enhance CO_2 formation, because deeper activated oxygenated peat layers did not substantially contribute to CO_2 emissions. In general, total C-release from D5 during summer droughts appeared to be lower compared to other peatland studies.

Exoenzymatic activities of the surface peat layer (0-10 cm) increased during the water table drawdown in 2007 by a factor of 3.3 to 6.9 up to levels of activities measured during pumping in 2006 (Figure 4). Exoenzymatic activities can be reactivated in soils by more favorable redox or pH conditions [*Pind, et al.*, 1994]. The pH effect can be ruled out, because pH of the soil water varied only from 4.3 to 4.9 during the manipulations, and exoenzymatic activities were not affected in the pH range from 4 to 7. Thus, peat oxygenation might have stimulated exoenzymatic activities e.g. by a decreased solubility of inhibitory metals due to a chemical oxidation [*Pulford and Tabatabai*, 1988]. Phosphate might be bound in organic matter or be adsorbed at Fe- or Al-precipitates especially under oxic conditions in this fen. The high hydrolytic activities for phosphatases and the absence of dissolved phosphate in the soil water suggest phosphate limitation in this fen despite the high amounts of total phosphorus in the solid phase (Table 1). Since the activity of phosphatases showed no preference for acid or alkaline conditions, the origin from either plant roots or from microorganisms [*Krämer and Green*, 2000] can not be distinguished.

Soil exoenzymes are stabilized by organic matter but are also inactivated or inhibited by phenolic compounds or clay particles [*McLaren*, 1975; *Freeman, et al.*, 2001; *Tietjen and Wetzel*, 2003]. The activity of phosphatases and β-glucosidases were inhibited in the presence of high amounts of added humic acids containing phenolic compounds. The concentration of phenolic compounds were low in the soil solution of oxygenated peat during the water level drawdown and rewetting 2007 but reached high concentrations in 10-20 cm depth. Phenolic compounds can be degraded via activated phenol oxidases under oxic conditions [*Freeman, et al.*, 2001]. However, we never observed any activity of phenol oxidases despite the overcoming of the oxygen constraints. Water table drawdown does also not affect phenol oxidase activities in a bog [*Freeman, et al.*, 1996]. Alternatively, phenolic compounds might have been eliminated from the soil solution by adsorption on freshly precipitated Fe(III) [*Gu, et al.*, 1994; *Kalbitz, et al.*, 2000]. Indeed, HCl-extractable Fe(III) increased near the surface at the end of pumping in 2006. In contrast, despite the penetration of oxygen in deep peat layers and the accumulation of Fe(III) at the end of pumping in 2006, exoenzymatic activities

were not enhanced in 10-30 cm depth. Thus, phenol oxidase activation or the removal of inhibitory compounds via adsorption was not clearly linked to an enhanced activity of hydrolytic exoenzymes responsible for peat degradation. Increased peat aeration, as a result of drought as predicted by climate-change models, might not eliminate this critical mechanism [*Freeman et al.*, 2001] restricting the re-release of CO_2 to the atmosphere in this fen.

Effect of drought and rewetting on source-sink functions

A strong increase in microbial activity, especially a microbially induced short-term flush in CO_2 emission, has been shown in mineral soils after rewetting [*Kieft, et al.*, 1987; *Fierer and Schimel*, 2003; *Iovieno and Baath*, 2008]. Underlying mechanisms are the release of physically protected organic matter by disruption of soil aggregates or dissolution from soil surfaces [*Appel*, 1998; *Denef, et al.*, 2001], the decomposition of dead microbial biomass killed by drying [*Bottner*, 1985], and the release of carbon substrates by microbial hypo-osmotic stress response [*Kieft, et al.*, 1987; *Fierer and Schimel*, 2003]. However, no increased basal soil respiration and anaerobic CO_2 formation rates could be determined even one day after rewetting of dried surface peat layers in 2006 (Figure 3). Despite the low groundwater level below the piezometer depth of 40 cm, the mean peat moisture declined only by 7 % during pumping in 2006 and reached minimum values of 60 to 82 % which appeared not to be sufficient for the mechanisms listed above. In contrast, in other studies mineral soils yield moisture contents after drying of 3 to 10 % [*Fierer and Schimel*, 2003; 2003; *Miller, et al.*, 2005; *Rey, et al.*, 2005; *Iovieno and Baath*, 2008].

Periods of long and extensive peat oxygenation lead to a reoxidation and accumulation of former reduced compounds to nitrate, Fe(III), and sulfate [*Regina, et al.*, 1996; *Devito and Hill*, 1999; *Dowrick, et al.*, 2006; *Paul, et al.*, 2006] which are available as alternative electron acceptors after oxygen depletion. The manipulated summer drought of 2006 yielded higher concentrations of nitrate and sulfate in the soil solution than the less extreme drought of 2007. Nitrate was depleted 19 days after rewetting apparently due to rapid denitrification, but sulfate concentrations still exceeded 100 µM even. Increased sulfate concentrations were observed after rewetting in the small stream that drained the wetland area (Weyer, C., manuscript in preparation, 2008) similar to the enhanced stream sulfate concentrations measured after a heavy rainfall in 2003 [*Küsel, et al.*, 2008]. Thus, increased weather extremes will strengthen the sink function of this fen for nitrate due to rapid removal by denitrification, but enhance its source function for sulfate.

In 2007, peat oxygenation during pumping led to an increase of Fe(III) near the surface (0-10 cm depth). This Fe(III) pool was nearly depleted 6 days after rewetting suggesting rapid microbial Fe(III) reduction. Rates of Fe(II) formation and final Fe(II) concentrations showed a positive correlation ($r^2 > 0.8$) suggesting that the amount of bioavailable Fe(III) was enhanced at low water level. In addition, Fe(II) formation rates paralleled the enhanced exoenzymatic activities and basal soil respiration rates in the upper 0-10 cm depth during pumping 2007 which point also to enhanced electron donor availability. Microbial Fe(III) reduction can account for up to 70 % of the anaerobic organic carbon mineralization in the upper 0-10 cm depth of this fen [*Küsel, et al.*, 2008]. Rewetting in 2006 yielded even higher concentrations of HCl-extractable Fe(III) down to 30 cm depth than Fe(III) measured after end of pumping. Thus, mixing of rainwater with deeper more reduced groundwater containing Fe(II) appears to be an important mechanism for the accumulation of Fe(III) near the surface. A delayed onset of CH_4 formation after rewetting was also observed when intact peat monoliths from this fen were subjected to drought and rewetting [*Knorr, et al.*, 2008]. Proportions of acetoclastic methanogenesis were independent from water table manipulations. The concept that CO_2 reduction becomes more important as the supply of labile organic compounds becomes depleted with soil depth [*Whiticar, et al.*, 1986; *Hornibrook, et al.*, 1997; *Chasar, et al.*, 2000] could not be supported similar to previous results [*Reiche, et al.*, 2008]. In general, C5 and D5 showed no net emissions of CH_4 to the atmosphere during the manipulation (Knorr, K. H., Goldberg, S., manuscripts in preparation, 2008).

Conclusions

Extreme summer droughts might not further enhance mineralization processes in this fen, because the upper most active peat layer appeared to be adjusted to drying and oxygenation, and deeper peat layers with increased enzymatic and respiratory activities did not substantially contribute to CO_2 emissions. The exoenzymatic activities did not support the enzymatic latch hypothesis that a shortage of oxygen locks up carbon in peatlands by restraining a single enzyme, the phenol oxidase, that restricts the re-release of CO_2 to the atmosphere. Drought followed by a heavy rainfall will not result in a CO_2 flush, because the high water holding capacity of peat prevent from a rapid dehydration during lowered water tables. However, the flow of reductants from organic matter decomposition will be shifted away from methanogenesis towards other anaerobic processes especially to nitrate and Fe(III)

reduction, whereas a part of the re-oxidized sulfur pool will be exported to nearby streams and affect water quality.

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EFFECT OF PEAT QUALITY ON MICROBIAL RESPIRATION AND METHANOGENESIS IN AN ACIDIC FEN

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Abstract

Availability of water and oxygen in peatlands plays a major role in the decomposition of stored peatland carbon. With climate change these parameters are expected to change and large amounts of carbon might be released from northern peatlands to the atmosphere. So far it is still an open question how peat quality can affect the consequential emission of relevant greenhouse gases like CO_2 and CH_4 from peatlands. Thus, we link the chemical composition of carbon-based compounds present in peat of an acidic fen (pH ~4.7) with anaerobic formation of CO_2 and CH_4 in zones from 0 to 40 cm depth and developed a fast and simple peat quality index to estimate the greenhouse gas potential of peat.

In general, CO_2 and CH_4 formation were highly spatially variable and depended not only on depth and sampling area but also on peat quality. Peat samples with a high methanogenic and respiratory activity had a quality index above 1.35 which states that the fraction of labile pyrolyzable organic matter (comparable with microbial easily available carbon substrates) obtained by thermogravimetry was above 35%. Curie-point pyrolysis-gas chromatography/mass spectrometry was used to characterize the chemical composition of the pyrolyzable fraction. The proportion of pyrolysis products from carbohydrates and lignin decreased parallel to respiration and methanogenesis with depth. In contrast pyrolysis products of lipids accumulated in the depth profile. These lipids derive from leave and root waxes, and are highly resistant to biodegradation. Our results suggest that undecomposed plant biomass that is still rich in carbohydrates and lignin is a prerequisite for CH_4 and CO_2 development from acidic fens.

Using the new quality index to estimate the greenhouse gas potential of peat in processes like peatland restoration, permafrost development under changing climate conditions or rewetting and thawing events represents a robust basis for modeling and calculating element cycles or trace gas fluxes from peatlands.

Keywords: Thermogravimetry (TG), Curie-point pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS), Fen, Soil, Anoxic incubation

Introduction

Peatlands maintain an imbalance between net primary production and decomposition leading to the storage of a large carbon (C) pool (Gorham 1991) due to the slow mineralization of plant biomass (Clymo 1983). Two factors that limit decomposition rates have been implicated. First, unfavorable environmental conditions for microorganisms involved in mineralization, i.e. low temperature, low pH, waterlogged peat, less or no availability of oxygen and an inhibitory effect of humic substances. Second due to the complexity of organic matter and a deficiency of nutrients the resources for peat microorganisms are low in availability. Thus, although peat soils represent a large C-pool (Gorham 1991), it was shown that the reduced quality of organic matter leads to a substrate limitation of the microbial metabolism because of a decreased bioavailability of organic carbon (Bridgham and Richardson 1992; Wagner et al. 2005).

Peatlands are also known to be sources for relevant greenhouse gases like CO_2 and CH_4 (i.e. Aselmann and Crutzen 1989; Charman et al. 1999). Atmospheric concentrations of both gases are increasing rapidly, with consequences for the future global climate (Houghton 2005). Measured emissions and formation rates of CO_2 and CH_4 demonstrate a high spatial and temporal variation between and also within peatland sites (i.e. Moore and Knowles 1990; Moore et al. 1990; Reiche et al. 2008b; Svensson and Rosswall 1984; Whalen and Reeburgh 1990). This variation results in part from sampling and measurement errors. However, the bulk of the variation is probably related to the fact that CO_2 and CH_4 emission from anaerobic soils is affected by numerous of biogeochemical factors, which are highly variable in space and time (Nilsson and Bohlin 1993). Factors such as temperature, oxygen availability, ground water level and type of vegetation were linked to gas emission rates from peatlands (Bridgham and Richardson 1992; Moore and Knowles 1990; Petrescu et al. 2008; Roulet et al. 1992a; Williams and Crawford 1984; Yavitt et al. 1987) and although there has been some success in relating and modeling water level and temperature to CO_2 and CH_4 emissions within particular systems (Harriss et al. 1982; Petrescu et al. 2008; Roulet et al. 1992b; Strack and Waddington 2007; Walter and Heimann 2000), these variables are insufficient for predicting emissions across a variety of peatlands (Whiting and Chanton 1993). Additionally, it was shown that concentrations of CO_2 and CH_4 in peat profiles are correlated not only with depth but also on degree of decomposition or botanical composition of the peat (Moore and Dalva 1997; Nilsson and Bohlin 1993). It is generally accepted that CO_2 and CH_4 formation, which are important indicators of total C mineralization, are also controlled by both the

quality and quantity of available organic matter present in peat (Bridgham and Richardson 1992; Christensen et al. 2003; Crozier et al. 1995; Reiche et al. 2008a; Reiche et al. 2008b; Valentine et al. 1994; Whiting and Chanton 1993; Yavitt and Lang 1990), however, so far a simple parameter to estimate the greenhouse gas potential of peatland is still missing.

In this study, we applied thermogravimetry (TG) to derive a quality index based on thermo-degradability properties of peat. A comparison of peat quality index with anaerobic CO_2 and CH_4 formation was used to understand the influence of the chemical peat composition on the extent of both processes. We hypothesized that a peat with a high quality index should have higher concentrations of easily available substrates for microbial uptake which enhances the anaerobic formation of CH_4 and CO_2. Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) analyses were used to identify the biological precursors of pyrolysis products present in peat samples obtained from an acidic fen.

Materials and methods

Peat sampling

Samples were obtained from an acidic fen (Schlöppnerbrunnen, fen area: 0.8 ha, pH 4.7) located in the northern Fichtelgebirge region in east-central Germany (50°7'54" N, 11°52'51" E, 700 m above sea level) as previously described (Reiche et al. 2008b). The mean annual precipitation between 1995-2006 approximated 953 mm and mean annual air temperature was 6.1°C. The Schlöppnerbrunnen fen has an average peat accumulation of about 50 cm and soil is Histosol on granite bedrock. Vegetation is dominated by *Carex canescens*, *Carex rostrata*, *Juncus effuses, Molinia caerulea,* and *Eriophorum vaginatum.* The ground water flows slightly through the fen from the north to the south (Paul et al. 2006), but fen site showed increased water saturation from north to south and from east to west due to a slight slope in the field site. Peat was sampled on four areas from the middle part to the southern part of the fen following the hydrological gradient. Areas were named C5, D5, sD4, M according to previous investigations (Reiche et al. 2008a; Reiche et al. 2008b). Peat obtained at C5 and D5 was dark brown to black in color and the degree of decomposition according to von Post's humification scale (Clymo 1983) was higher (moderately decomposed, H6-7) than for the brownish peat at sD4 and M (slightly to moderately decomposed H3-5). Peat samples from 0-40 cm depth were obtained using a peat corer with a diameter of 8 cm. Fresh plant litter was removed from the top and cores were separated in 10 cm depth segments (I: 0-10 cm,

II: 10-20 cm, III: 20-30 cm, IV: 30-40 cm). Peat samples were transported to the laboratory in airtight plastic bags at 4°C. Samples were processed within the same day.

Microcosm incubations and headspace gas determination

To study anaerobic soil respiration rates and formation of methane, 20 g (fresh wt peat) were placed into sterile 180 mL incubation flasks (Mueller & Krempel, Buelach, Switzerland) in three replicates under a continuous flow of sterile argon (anoxic incubations). Flasks were closed with rubber stoppers and screw-caps, and were incubated in the dark with an initial overpressure of approximated 100 mbar. Headspace concentrations of CO_2 and CH_4 were determined every 2 to 3 days during an incubation period of 31 days at 15°C. Headspace gases were measured with Hewlett Packard Co. 5980 series II gas chromatographs according to Reiche et al. (2008b). A sample volume of 100 µl was obtained from the headspace of microcosms after shaking them to release gas trapped inside the peat. CO_2 was separated with a Chromosorb 102 (60/80 mesh, Alltech, Unterhaching, Germany) column (length, 2 m; inner diameter, 3.2 mm). Analysis was carried out by a thermal conductivity detector. The determination of CH_4 was conducted with a molecular sieve 13X (60/80 mesh, Alltech, Unterhaching, Germany) column (length, 2 m; inner diameter, 3.2 mm) and analyzed with a flame ionization detector.

Analytical techniques

Field fresh duplicate peat samples were dried at 105°C for 24 hours to determine water contents (WC) and than burned at 500°C for 4 hours to calculate the ash content as loss on ignition (LOI). Total P, Fe, Al, Mg, Ca, S, H, N and C of dried (60°C for 48 hours) and milled (Mixer Mill MM301, Retsch, Germany) soil samples were analyzed with an elemental analyzer (vario EL, Elementar, Germany), by flame atomic absorption spectrometry (Perkin Elmer, 3300, USA) or photometrically (Varian, Cary 1E, USA) after acid digestion as previously described (Reiche et al. 2008b).

Approximately 5 mg of each dried and milled peat sample (in two replicates) was analysed by thermogravimetry (Mettler Toledo, TGA / SDTA 851e, Switzerland) (TG) (Pope and Judd 1977) to measure the degradability at increasing temperatures (0.17°C s^{-1}) under a continuous flow of argon from 60-850°C followed by a final combustion under oxygen at 850°C (Rubino et al. 2007).

Curie-point pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS) (Gleixner et al. 1999) with approximately 0.7 mg of selected peat samples (C5 I and III, D5 1, II and IV, M I and IV; two replicates each) was used to determine major C-based compounds in peat. Peat samples were selected with respect to their difference in the peat quality index (described below). Py-GC/MS is a powerful technique to distinguish between plant derived "biodegradable" and more "humified" compounds. Pyrolysis products like furanes, substituted phenols or alkanes that derive from carbohydrates, lignin or lipids, respectively, indicate the presence of plant material whereas pyrolysis products like benzol, phenol or naphthalene present highly humified organic material (Gleixner et al. 1999; Rubino et al. 2007). Pyrolysis was carried out under helium for 9.9 s at 500°C with a Curie point Pyrolyzer 0316 (Thermo Fisher, USA). Volatile pyrolysis products were separated by gas chromatography (HP 5890, Germany) with a BPX5 capillary column (length, 60 m; inner diameter, 0.32 mm; film thickness, 1 µm; SGE, Germany) and analyzed by ion trap mass spectrometer (Thermo Fisher, GCQ, USA) (Steinbeiss et al. 2006).

Calculations and statistics

Rates for CO_2 and CH_4 formation were determined from the linear increase of headspace and dissolved gas concentrations. Mean formation rates were calculated from the three replicates. Peat samples were then grouped according to their methanogenic and respiratory activity on the basis of hierarchical cluster analysis. The grouping was carried out using the Ward method, based on the Euclidean squared distances (SPPS 15.0, SPPS Inc., Chicago, Illinois, USA). Pearson's correlation coefficients (r) were calculated to test anaerobic CO_2 and CH_4 formation for their correlation with chemical peat parameters (SPPS 15.0, SPPS Inc., Chicago, Illinois, USA).

The chemical characteristics of each peat sample could be compared by comparing the mass loss of three distinct temperature intervals obtained with TG technique. Three important temperature intervals were selected using variance analyses of mass loss spectra (mean of two replicats). The first temperature interval ranged from 205-360°C (rapid mass loss due to labile particulate organic matter; POM_{labile}), the second from 585-630°C (slow mass loss due to more recalcitrant particulate organic matter; $POM_{recalcitrant}$), and the third one was the sudden combustion under oxygen at 850°C (highly humified and inert particulate carbon compounds; POM_{inert}). Mass loss was normalized to total pyrolyzed matter.

The peat quality index was calculated as ratio between the sum of mass loss of the first and second interval with the third one:

$$QI_{peat} = \frac{POM_{labile} + POM_{recalcitrant}}{POM_{inert}}$$

In principle, the higher the quality index the higher the quantity of labile organic matter. Evaluation of mass spectra was done according to Kracht and Gleixner (2000) and by comparison with spectral databases like Wiley 6.0 (McLafferty 2001), the National Institute of Standards and Technology (NIST 2002) and the Integrated Spectral Data Base System for Organic Compounds (AIST 2001). Means of two replicates from the mass list of pyrolysis spectra, the relative abundances of representative precursor groups (lipids, carbohydrates, lignin and unspecific C-based compounds), normalized to 1 mg pyrolyzable sample, were calculated as the summed peak areas of individual pyrolysis products belonging to the same precursor group (Table 1).

Table 1. Retention time, peak identification, precursor groups and mass spectrometric characteristics of major pyrolysis products generally present in selected peat samples (C5, D5, M, according to Figure 4) obtained over depth (0-40 cm) of an acidic fen

Retention time (min)	Identified compound	Precursor[a]	Molecular weight (g mol^{-1})	Base peak (*m/z*)[b]	Characteristic fragments (*m/z*)
10.6	2-Methylfuran	ch	82	81	82, 53
13.3	Benzene	us	78	78	77, 58, 51
17.9	Toluene	us	92	91	92, 65, 50
19.5	2[3H]Furanone	ch	84	55	84, 54
20.9	2-Furaldehyde	ch	96	95	96, 39, 37
22.1	Dimethylbenzene	us	106	91	106
23.1	Ethylbenzen/Styrene	us	106	91	78
25.8	5-Methyl-2-furaldehyde	ch	110	109	110, 53, 50
26.2	Phenol	us	94	94	66
27.4	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	ch	114	114	58, 85, 57
28.9	2-Methylphenol	us	108	108	107
29.6	3,4-Methylphenol	us	108	107	108, 77, 79
30.2	2-Methoxyphenol	lg	124	124	109, 81

32.5	p-Ethylphenol	lg	122	107	77, 122
33.4	4-Methyl-2-methoxyphenol	lg	138	138	123
33.9	n-C12/C13 alkane/alkene	li	n.d.[c]	57	85, 70
34.3	4-Vinylphenol (4-ethenylphenol)	lg	120	120	91
35.9	4-Ethyl-2-methoxyphenol	lg	152	137	152
37.2	4-Vinyl-2-methoxyphenol	lg	150	150	135, 107
39.9	4-Formyl-2-methoxyphenol	lg	152	151	152
40.8	trans-4-(2-propenyl)-2-methoxyphenol	lg	164	164	116
42.1	4-Acetyl-2-methoxyphenol	lg	166	151	166
42.7	Levoglucosane	ch	162	60	73
43.6	4-Vinyl-2,6-dimethoxyphenol	lg	180	180	165, 137
45.6	n-C17 alkene	li	n.d.	55	69, 83
46.3	n-alkene	li	n.d.	111	70, 55, 69
47.0	Trans-4-(2-propenyl)-2,6-dimethoxyphenol	lg	194	194	131
47.8	n-C18 alkene	li	n.d.	55	69, 83
47.9	n-C18 alkane	li	n.d.	57	71, 85
47.9	4-Acetyl-2,6-dimethoxyphenol	lg	196	181	196
49.9	n-C19 alkene	li	n.d.	55	69, 83
49.9	n-C19 alkane	li	n.d.	57	71, 85
51.8	n-C20 alkene	li	n.d.	55	69, 83
51.9	n-C20 alkane	li	n.d.	57	71, 85
53.8	n-C21 alkane/alkene	li	n.d.	55	57, 69, 71
55.6	n-C22 alkane/alkene	li	n.d.	55	57, 69, 71
57.3	n-C23 alkane/alkene	li	n.d.	55	57, 69, 71
59.0	n-C24 alkane/alkene	li	n.d.	55	57, 69, 71
61.0	n-C25 alkane/alkene	li	n.d.	55	57, 69, 71
63.1	n-C26 alkane/alkene	li	n.d.	55	57, 69, 71

[a] ch=carbohydrates; lg=lignins; li=lipids; us=unspecific

[b] mass-to-charge ratio

[c] could not be determined

Results

Chemical properties of peat

The C content of peat samples obtained from C5 and D5 increased from 36% in 0-10 cm depth to more than 50% in 30-40 cm depth, respectively (Table 2). In contrast, amount of total C decreased with increased depth at sD4 and M. The total amount of N decreased at all sampling areas over depth and concentrations yielded 0.5 to 2.1%. Corresponding C:N ratios were lowest in upper peat compared with depth below and ranged from 18 to 44% in the depth profile of 0-40 cm. Content of H and loss on ignition ranged from 1.4% to 6.7% and from 31% to 92%, respectively, and increased at C5 and D5 with an increase in depth, but decreased over depth at the southern areas sD4 and M (Table 2). Highest amounts of total Fe and Al were obtained in the first zone of areas D5, sD4 and M yielded up to 36.4 and 43.8 mg g (dry wt peat)$^{-1}$, respectively. Total concentrations of Mg, Ca, P and S were mostly evenly distributed over depth and sampling areas, and mean values approximated 0.8, 0.3 and 1.3 mg g (dry wt peat)$^{-1}$ and 0.3%, respectively (Table 2).

Microbial respiration and methanogenesis

Peat soil anaerobic CO_2 formation and methanogenic activities varied between sampling area and depth (Table 3). Microbial respiration over depth was highest at sampling area M compared with C5, D5 and sD4 peat samples. In general, microbial respiration decreased strongly with an increasing depth at all sampling areas. Respiration rates in the upper most peat zone yielded up to 12.7 µmol CO_2 g (dry wt peat)$^{-1}$ and zones below yielded less than 1.2 µmol CO_2 g (dry wt peat)$^{-1}$ (Table 3). In general, peat obtained from the more southern areas sD4 and M showed a potential CH_4 formation with an apparent delay of 2 or 7 days in the depth of 0-40 cm, but also initial methanogenesis could be detected below 20 cm depth at sD4. Methane formation rates ranged between 0.04 and 2.11 µmol CH_4 g (dry wt peat)$^{-1}$ with a notable increase in the zone of 0-10 cm and 10-20 cm of sD4 and M, respectively (Table 3). In 0-10 cm depth peat obtained from C5 and D5 showed a potential formation of CH_4, which started after an incubation of approximately 8 and 12 days, respectively. In depth below, no formation of CH_4 occurred during the prolonged incubation of 30 days.

Peat samples were grouped according to their methanogenic and respiratory activity on the basis of hierarchical cluster analysis. Thus, rates for CH_4 and CO_2 formation below 0.1 and 1.2 µmol g (dry wt peat)$^{-1}$ d^{-1}, respectively, were indicated as inactive peat ($peat_{inactive}$) and rates above as active peat ($peat_{active}$) (Figure 1).

Table 2. Chemical characteristics in peat obtained from an acidic fen (pH 4.7) along a hydrological gradient (from the middle to the southern part C5 → D5→ sD4 → M) over depth (I: 0-10 cm, II: 10-20 cm, III: 20-30 cm, and IV: 30-40 cm) in November 2006

sample	WC[a]	LOI[b]	P_{total}	Fe_{total}	Al_{total}	Mg_{total}	Ca_{total}	S_{total}	H_{total}	N_{total}	C_{total}	C/N
depth	(%)		(mg g [dry wt peat]$^{-1}$)					(%)				ratio
C5 (I)[c]	80.3	69.6	1.8	9.5	7.2	0.9	0.2	0.3	4.5	2.0	36.2	18.1
C5 (II)[c]	81.7	83.3	1.5	5.9	4.1	0.3	0.1	0.3	5.4	2.1	47.3	22.5
C5 (III)[c]	81.6	88.2	0.8	6.2	3.3	0.3	0.3	0.2	5.5	1.5	51.5	34.3
C5 (IV)[c]	85.7	85.9	1.0	3.2	2.3	0.5	0.2	0.2	6.0	1.2	50.4	42.0
D5 (I)[c]	87	74.6	1.5	36.4	43.8	0.7	0.6	0.3	4.5	1.7	36.4	21.4
D5 (II)[c]	76.4	62.3	1.2	10.4	6.7	0.8	0.2	0.2	4.4	1.3	37.6	28.9
D5 (III)[c]	79.8	91.6	0.8	5.6	4.3	0.2	0.3	0.3	6.7	1.3	55.3	42.5
D5 (IV)[c]	84.4	85.4	1.0	4.7	3.4	0.5	0.2	0.3	6.0	1.3	51.0	39.2
sD4 (I)	90.9	77.7	1.4	28.7	20.2	0.6	0.3	0.4	4.5	1.7	39.0	22.9
sD4 (II)	90.3	74.8	1.5	5.7	2.8	1.0	0.3	0.5	4.7	1.4	37.7	26.9
sD4 (III)	78.6	52.1	1.3	6.9	2.1	1.6	0.2	0.3	3.4	1.0	29.4	29.4
sD4 (IV)	61.8	31.3	1.3	6.9	1.8	2.3	0.1	0.1	1.4	0.5	21.9	43.8
M (I)	91.2	86.3	1.3	19.9	15.9	0.4	0.4	0.3	5.3	1.8	43.1	23.9
M (II)	93.3	85.0	1.5	8.8	7.8	0.6	0.3	0.4	5.3	1.5	41.6	27.7
M (III)	92.9	82.5	1.4	4.5	3.2	0.6	0.3	0.6	5.1	1.4	41.5	29.6
M (IV)	85.9	68.8	1.5	7.1	3.8	1.1	0.3	0.5	4.6	1.3	37.6	28.9

[a] water content

[b] loss on ignition

[c] some data were obtained from Reiche et al. (2008a)

Table 3. Anaerobic CO2 formation, methane formation, and onset of methanogenesis in peat obtained from an acidic fen (pH 4.7) along a hydrological gradient (from the middle to the southern part C5 → D5→ sD4 → M) over depth (I: 0-10 cm, II: 10-20 cm, III: 20-30 cm and IV: 30-40 cm) in November 2006 (n=3)

Sample	CO_2 formation	CH_4 formation	Onset of CH_4 formation
	(μmol g [dry wt peat]$^{-1}$ d^{-1})		(day)
C5 (I)[a]	3.7	0.14	~8
C5 (II)[a]	1.0	0.00	n.a[b]
C5 (III)[a]	0.8	0.00	n.a.
C5 (IV)[a]	0.8	0.00	n.a.
D5 (I)[a]	12.7	0.32	~12
D5 (II)[a]	1.2	0.00	n.a.
D5 (III)[a]	0.9	0.00	n.a.
D5 (IV)[a]	0.6	0.00	n.a.
sD4 (I)	9.7	1.25	~5
sD4 (II)	1.7	0.32	~2
sD4 (III)	0.7	0.08	1
sD4 (IV)	0.1	0.04	1
M (I)	8.9	0.38	~7
M (II)	4.9	2.11	~2
M (III)	2.3	0.80	~2
M (IV)	0.8	0.07	~2

[a] some data were obtained from Reiche et al. (2008a)

[b] no methanogenic activity within 31 days of incubation

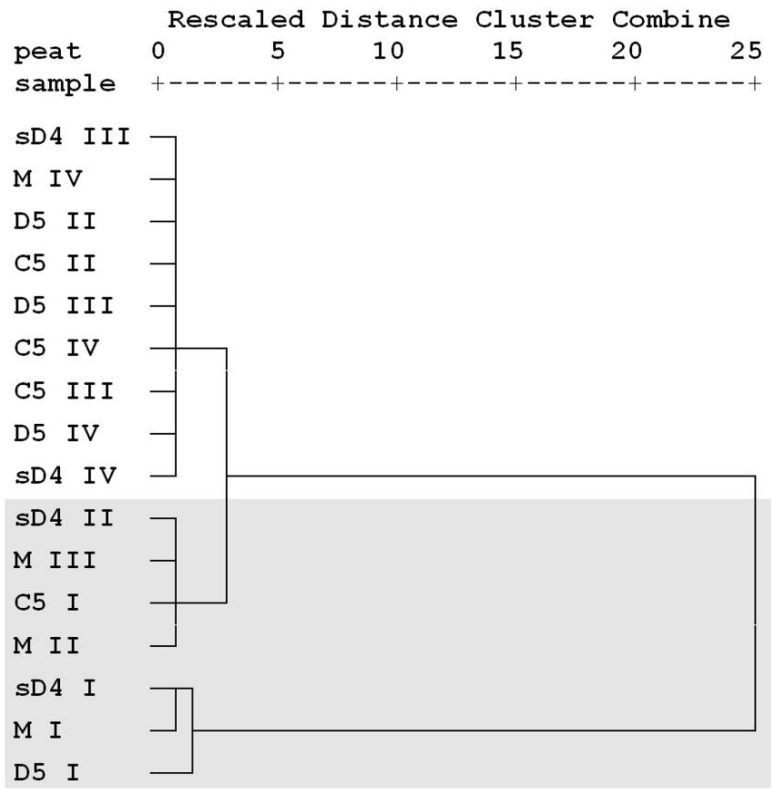

Figure 1. Grouping of peat samples according to their peat quality index and methanogenic and respiratory activity on the basis of hierarchical cluster analysis. The dendrograms were carried out using the Ward method, based on the Euclidean squared distances. Samples were obtained at different areas along a hydrological gradient from an acidic fen (from the middle to the southern part C5 → D5→ sD4 → M) over depth (I: 0-10 cm, II: 10-20 cm, III: 20-30 cm, IV: 30-40 cm). Active peat samples are highlighted (grey box)

Major C-based peat compounds and peat quality

The proportion of pyrolyzable labile organic matter (POM_{labile}) obtained with TG was highest in the upper zone (0-10 cm) at all sampling areas and yielded 38-43%. Lowest zones (30-40 cm) yielded 23-35% (Figure 2). Highest proportion of POM_{labile} in depth zone 10-30 cm was found at area M (41-44%). Pyrolyzable recalcitrant organic matter ($POM_{recalcitrant}$) reached 2.1-3.2% in the first peat zone of D5, sD4 and M, which was twice as high as in samples obtained from C5 or depth below 10 cm (Figure 2). In contrast, the percentage of pyrolyzable inert carbon compounds (POM_{inert}) increased with depth from approximately 25% to more than 28%.

Calculating peat quality index with respect to these three categories yielded values of 0.8 to 1.8. The index was highest in peat samples of the upper most peat zone compared with corresponding zones below (Figure 3). At sD4 and M high index was also observed up to 30 cm depth. Quality index was lowest for peat samples below 10 cm depth of C5 and D5.

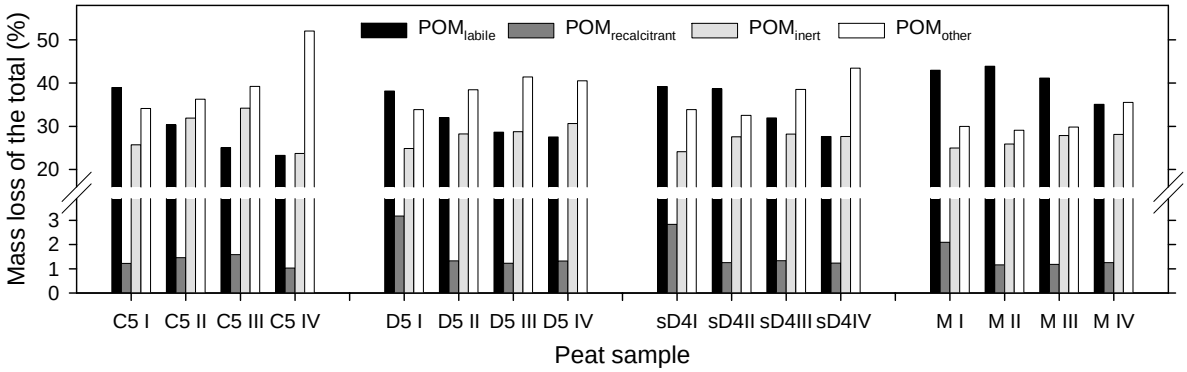

Figure 2. Percentage of mass loss during thermogravimetry analyses (n=2) in three temperature intervals corresponding to labile carbon (POMlabile: 205-360°C), recalcitrant carbon (POMrecalcitrant: 365-480°C) and inert carbon (POMinert: 850°C + oxygen) with respect to total pyrolysable organic matter. Mass loss which is not explained by these three temperature intervals is expressed by POMother. Peat was obtained from different sampling sites of an acidic fen along a hydrological gradient (from the middle to the southern part C5 → D5→ sD4 → M) over depth (I: 0-10 cm, II: 10-20 cm, III: 20-30 cm and IV: 30-40 cm)

The major Py-GC/MS products of all peat samples and precursor classes according to the molecule each Py-GC/MS product was generated from are given in Table 1. The number of peaks detected during the pyrolysis process increased over depth from 46 (0-10cm) to 64 (20-30cm) at C5 and from 44 (0-10 cm) to 80 (30-40cm) at D5 (data not shown). Peaks at sampling area M amounted 56 but no difference between 0-10 cm and 30-40 cm depth occurred. Low retention times between 10.8 and 27.3 min in the chromatogram of the Py-GC/MS were indicative for toluene, furan, furaldehyde, phenol and benzene derivates (Table 1). A "lignin region" between 28.9 and 43.6 min was dominated by methylphenol and methoxyphenol derivates. Compounds with high retention time, 45.6 to 63.2 min, and higher molecular weight dominated a "lipid region", e.g. n-alkens and n-alkans with a chain length of more than C17. The contribution of carbohydrates as calculated from the peak area of carbohydrate pyrolysis products decreased from 19% to 7% and from 16% to 6% with increasing in depth at C5 and D5, respectively (Figure 4). Carbohydrates at the most southern sampling area M reached 17% and was constant over depth. The contributions of lipids to the total pyrogram increased in deeper zones of D5 and M, whereas lignin pyrolysis products decreased like pyrolysis products from carbohydrates with depth. Lipid and lignin contribution to the pyrogram of C5 samples reached approximately 34% and 25% in depth I (0-10 cm) and III (20-30 cm), respectively. Total pyrolyzable in relation to dry matter approximated 48% in peat samples obtained from 20-40 cm depth and 64, 48, 59% in upper peat of C5, D5 and M, respectively (data not shown).

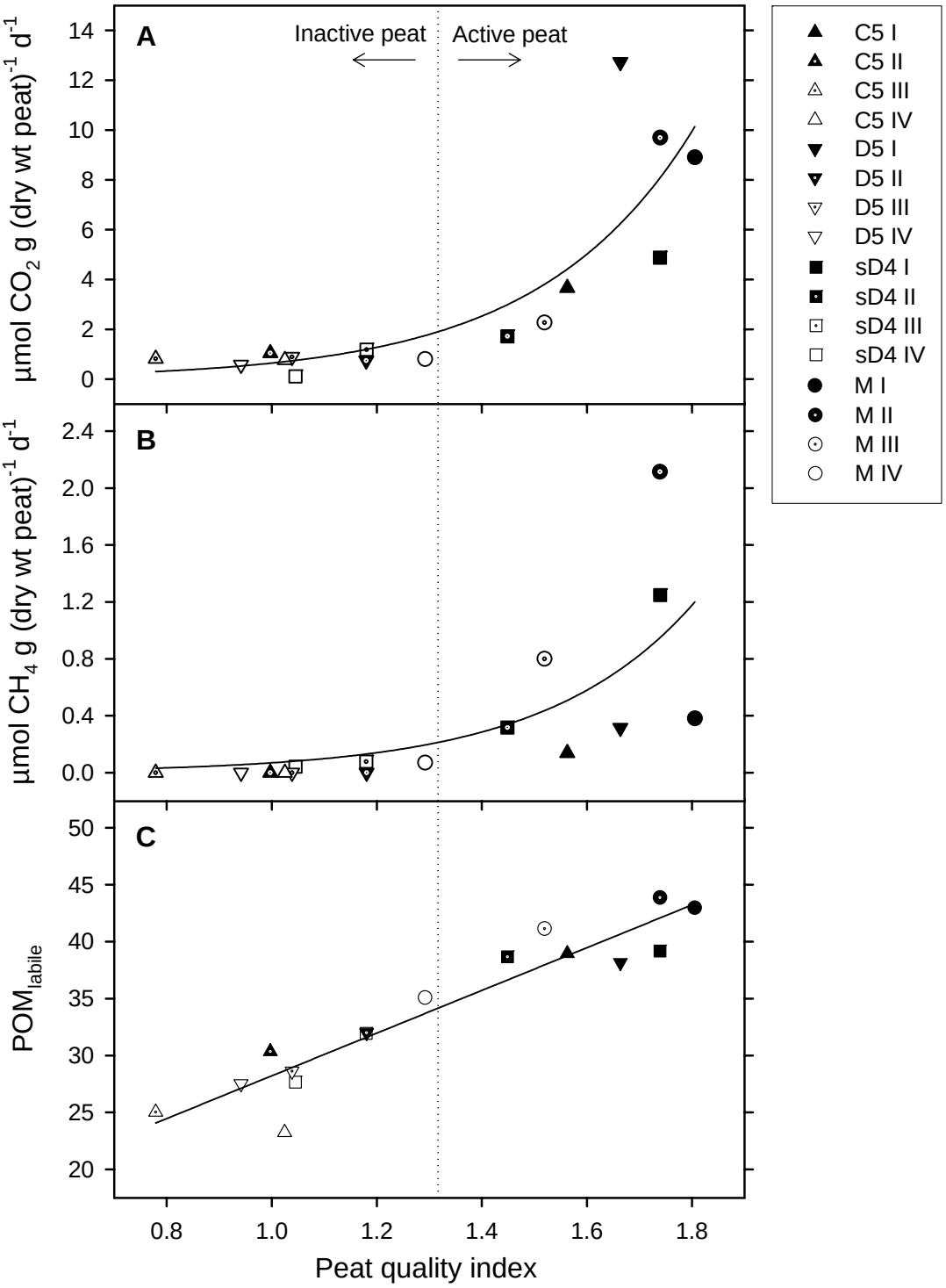

Figure 3. Correlation of peat quality index (ratio between sum of labile and recalcitrant C-based compounds and inert carbon compounds obtained with thermogravimetry analyses, n=2; POMlabile+POMrecalcitrant/POMinert) with rates of microbial respiration (A), formation of methane (B) and proportion of labile particulate organic matter (POMlabile) (C). Peat was sampled at sampling sites along a hydrological gradient of an acidic fen (from the middle to the southern part C5 → D5→ sD4 → M) over depth (I: 0-10 cm, II: 10-20 cm, III: 20-30 cm and IV: 30-40 cm). The best fit equation for microbial respiration, methanogenesis and POMlabile with peat quality is expressed by: y=0.025e3.19x (r2=0.71, p<0.01), y= 0.002e3.54x (r2=0.49, p<0.01) and y=18.75x+9.5 (r2=0.89, p<0.01), respectively

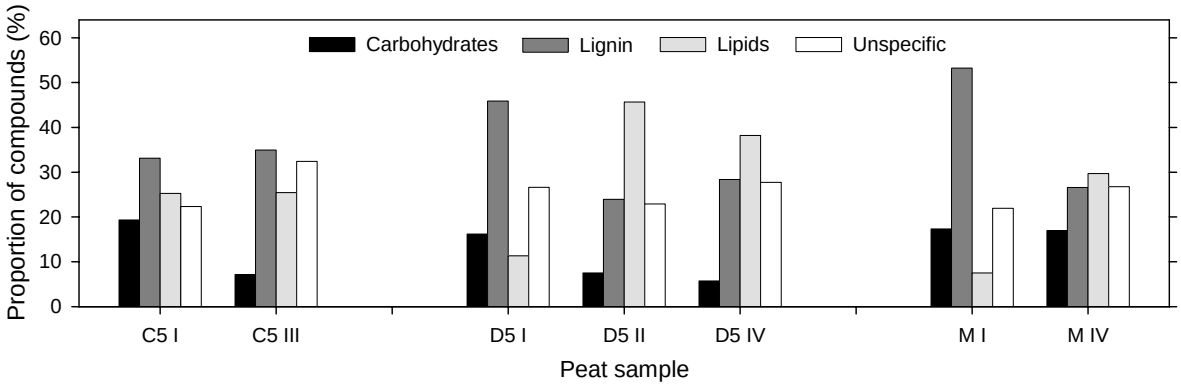

Figure 4. Relative proportion (% of total amount of pyrolysable compounds) of identified compounds grouped in relevant precursor classes of chemical compounds (carbohydrates, lignin, lipids and unspecific C-based compounds) of different peat samples obtained from an acidic fen (C5: middle part, D5: more southern part, M: most southern part) over depth (I: 0-10 cm, II: 10-20 cm, III: 20-30 cm and IV: 30-40 cm). Compounds were identified using Curie-point pyrolysis-gas chromatography/mass spectrometry (n=2)

Discussion

Peat composition

In many well drained terrestrial soils degradation of organic matter basically proceeds with molecular oxygen as electron acceptor. This allows the microorganisms to break down complex organic matter, i.e. lignin and waxes. In contrast, degradation in peatlands based mostly on anaerobic metabolism pathways which are less energy efficient from a thermodynamic point of view. The high proportion of organic matter in all peat samples suggests that their degradability is decreased under the water logged and therefore anoxic conditions. Water saturation is an important factor controlling peat degradation (Clymo 1984) and thus, variations of water logging i.e. due to a slope at the field site or natural fluctuating water tables during dry and wet weather conditions seem to affect the quality of peat (Belyea 1996; Hogg et al. 1992; Reiche et al. 2008b). Peat of southern water logged sampling areas sD4 and M was less degraded and amounts of POM_{labile} in depth below 10 cm was high in comparison with samples obtained from more hydrological and redox instable areas C5 and D5. There mean peat quality index was 1.3 times lower than in peat samples of sD4 and M (Figure 3).

The proportion of POM_{labile} and $POM_{recalcitrant}$ in upper peat zones (Figure 2) seemed to be increase due to fresh and therefore less decomposed plant litter inputs, i.e. roots and leaves of the growing vegetation. There are indications that botanical origin of plant litter influences

the chemical composition and degradability of peat (Moore et al. 2007). For instance, *Carex* peat contains much less cellulose and hemicellulose compared to *Sphagnum* peats (Bohlin et al. 1989). Both kind of C-based compounds are likely substrates for hydrolytic fermentation (Zeikus 1983) and thus, yielding different amounts of precursors available for anaerobic CO_2 formation and methanogenesis. It was also shown that vegetation may increase the pool of easy available carbon substrates such as carbohydrates and amino acids through the leakage of exudates from living plant roots (Grayston et al. 1996; Yan et al. 2008). In contrast, increased number of detectable peaks obtained with Py-GC/MS at areas C5 and D5 affirm a higher complexity of C-based compounds in peat of lower zones and also increased proportion of unspecific carbon in depth below 10 cm was indicative for the more humified peat.

In general, in all pyrograms from peat samples, lipids, lignin, and to a lesser extent carbohydrates, were the major C-based pyrolysis products (Figure 4). Several studies have shown that aliphatic biopolymers are highly resistant to biodegradation and can be also well preserved in soils (Gleixner et al. 2001; Otto and Simpson 2006; Winkler et al. 2005). Thus and due to mostly anoxic conditions high amounts of long-chain lipids accumulated in deeper peat zones (Table 1 and Figure 4). The high proportion of linear alkane/alkene peaks in our Py–GC/MS data in peat zones below 10 cm at D5 and M suggests that aliphatic polymer material is an important part of the lower peat organic matter (Table 1 and Figure 4). Alkanes/alkenes are common compounds that derived from plant aliphatic polymers such as leave and root waxes, like cutin and suberin derived polymers (Gleixner et al. 2001; Kögel-Knabner 2002; Nierop 1998; Nip et al. 1986; Tegelaar et al. 1995; Tegelaar et al. 1993).

Content of pyrolysis products of carbohydrates in deeper peat accounted more then 30% of the initial amount obtained in upper peat and almost no difference in the contribution of carbohydrate related peaks was observed. Carbohydrates are known to be recycled or newly formed in soils during decomposition (Gleixner et al. 2002) and this might also happen in peat (Kracht and Gleixner 2000).

In general, high contents of LOI and total C (Table 2) let expect that most of the C is of an organic origin but lower proportion of pyrolyzable matter than LOI assume that only a part of the present organic compounds could be analyzed by Py-GC/MS. However, this analyzable part of peat organic matter may contain thermal labile compounds easily available for microbial degradation and formation of CO_2 and CH_4.

Microbial respiration and methanogenesis

CO_2 and CH_4 formation were highly spatially variable at the fen site and depended not only on depth but also on the sampling area. Spatial heterogeneity of geochemical conditions and microbial activities are often described in peatlands (Moore et al. 1990; Nilsson and Bohlin 1993) and also for this fen (Paul et al. 2006; Reiche et al. 2008b). CO_2 formation rates were increased in upper peat of all areas suggesting more favorable conditions for microorganisms than in depth below (Reiche et al. 2008a). In general, microbial activities are known to be most active in peat zones of the first few cm compared with deeper zones. Especially rates of microbial respiration and exoenzymatic activities reach highest activities (Freeman et al. 1995; Reiche et al. 2008a; van den Pol-van Dasselaar and Oenema 1999; Wright and Reddy 2001). Positive correlation ($p<0.01$) of CO_2 formation rates with total amounts of Fe ($r=0.95$), Al ($r=0.92$) and Ca (r=0.77) were found but not for WC, LOI, and total P, Mg, C, H, N and S ($p>0.05$) suggest, that especially Fe(III)-reducing activities in the upper most peat layer may contribute to anaerobic CO_2 formation (Reiche et al. 2008a). The positive correlation found for Al and Ca should not be connected with the anaerobic CO_2 formation, but rather co-precipitation with Fe(III)-oxides due to oxygenation events of the first peat zone may be discussed.

Formation of CH_4 was alspositively correlated with the peat WC ($r=0.58$, $p<0.05$). The high potential rates of methanogenesis at both southern areas sD4 and M, the short prolonged lag phase compared with C5 and D5 and the spontaneous formation of CH_4 in depth below 20 cm in peat sampled at sD4 (Table 3) were indicative for more water saturated conditions and thus, for more reduced conditions within the peat. A positive correlation of water table depth on methanogenesis has generally been found in peatlands (Matthews and Fung 1987; Moore and Dalva 1997; Roulet et al. 1992b). Despite, H_2, CO_2 and acetate are omnipresent intermediates in the anaerobic degradation of organic matter serving as main substrates for methanogenic archaea (Zinder 1993) peat below 10 cm obtained at the C5 and D5 areas was inactive in methanogenesis during an incubation period of 31 days. A decrease in CH_4 production with an increase in depth was previously described for other peatlands (Chow et al. 2006; Hughes et al. 1999; van den Pol-van Dasselaar and Oenema 1999). Methanogenesis rates of peat zones active in CH_4 formation were in a range reported for boreal peatlands (Bergman et al. 2000; Galand et al. 2005; Metje and Frenzel 2007; Rooney-Varga et al. 2007). In general wetland CH_4 emissions are thought to comprise around 80% of the total natural CH_4 sources, which are estimated to be around 250 Tg (Reay and Hughes 2006). However, in our study only few zones yielded high methanogenesis rates, indicating that CH_4 production is

not a significant pathway of carbon flow in this fen. This finding suggests that peatland sites with similar characteristics may contribute less to the global natural CH_4 emission than assumed.

Link of peat quality to microbial activity

The most likely factor responsible for differences in peat anaerobic CO_2 and CH_4 formation between the four sampling areas was the quality of peat organic matter. It is widely accepted that the quality of organic matter is one of the factors controlling the rate of organic matter mineralization (Bridgham and Richardson 1992; Crozier et al. 1995; Valentine et al. 1994; Wagner et al. 2005; Whiting and Chanton 1993; Yavitt and Lang 1990). Many quality indexes were proposed in the past, to reveal how difficult it is for organic matter to be biodegraded by microorganisms. Nonetheless, no common definition or a widely accepted quantitative index of "quality" exists (Rubino et al. 2007). Thus, the general definition that an organic matter high in quality may have a fast decay rate is not sufficient enough.

The ratio of C to N concentration (C:N ratio) or the ratio of lignin to N concentration (lignin:N ratio), for instance, have frequently been used as an index of litter quality (Enriquez et al. 1993; Gholz et al. 2000; Moore et al. 2007; Taylor et al. 1989; Valentine et al. 1994) and also in Canadian peatlands, lignin:N ratio provided a modest explanation of decomposition rate (Moore et al. 2005). Our CO_2 formation rates correlated negatively with the relevant C:N ratios (r=-0.60, p<0.05), but did not with lignin:N ratios and no correlation of CH_4 formation rates with their corresponding C:N or lignin:N ratios was found. However, the less C:N ratio in the first 10 cm (Table 2) confirmed that the quality of peat composition seemed to be better than in depth below (Artz et al. 2008; van den Pol-van Dasselaar and Oenema 1999). The positive correlation between formation of CO_2 (r=0.85, p<0.01) and CH_4 (r=0.92, p<0.05) with the content of lignin and the negative correlation between formation of CO_2 (r=-0.83, p<0.01) and CH_4 (r=-0.90, p<0.05) with the content of lipids suggest that less decomposed plant biomass that is still rich in lignin and poor in lipids present at area M and in upper peat of areas C and D is a prerequisite for CO_2 and CH_4 development from this fens. High rates of CO_2 and CH_4 formation at the sD4 and M areas could be also explained by the low degree of peat decomposition according to the von Post's humification scale (Clymo 1983). However, this value was not sufficient enough to explain the small scale spatial heterogeneity of both formation rates at areas C and D.

In order to have a rapid way to compare microbial respiratory activities with reproducible chemical properties of peat, our results let us suggest that peat quality can be

described as ratio between the sum of labile and recalcitrant carbon compounds with proportion of highly humified inert C-based compounds obtained with the TG-technique. In principle, the lower the quality index the higher the quantity of inert C-based compounds in the peat. Correspondingly, a peat with a high quality index showed higher concentrations of easily biodegradable C-based compounds.

Anaerobic metabolism pathways, mainly responsible for degradation in peatlands, can be indirectly evaluated by determination of microbial CO_2 and CH_4 formation rates (Bridgham and Richardson 1992). That means a peat high in microbial activity should have easily available substrates for microbial uptake. The thermal degradability of peat obtained with TG might not agree with microbial availability, however, assuming that the fraction of POM_{labile} represents easily available substrates, i.e. from less decomposed plant litter or root exudates, peat was active in CO_2 and CH_4 formation when the proportion of this fraction was above 35%. Correlating TG with Py-GC/MS data carbohydrates seem to be a relevant part of this POM_{labile} (r^2=0.78) (Figure 2 and Figure 4). In addition, there are suggestions that CO_2 reduction becomes increasingly important as the supply of labile organic compounds becomes depleted with depth in soils (Chasar et al. 2000; Hornibrook et al. 1997; Whiticar et al. 1986). Amendment experiments indicate that the poor substrate quality of highly decomposed, humified peat limits both CO_2 and CH_4 production rates, even though the peat was 95% organic matter (Bridgham and Richardson 1992). In general, anaerobic CO_2 formation rates strongly decreased with depth at our fen site indicating the importance of fresh plant tissue as source of POM_{labile}.

At all sampling areas, a strong relationship was found between microbial activity and peat quality index (Figure 3) but it is still an open question how long-term stable a present peat quality with respect to the potential CO_2 and CH_4 formation is. There are suggestions from mesocosm experiments that a change in size and/or quality of the labile carbon pool can be occur in a relatively short period of time (Keller et al. 2004).

Conclusion

Our results demonstrated the possibility of a small scale spatial heterogeneity of chemical peat composition and microbial activities over space and depth of a fen site. Thus, adequate assessment of the contribution of peatlands to the global CO_2 and CH_4 budget requires not only field measurements of gas fluxes over the complete season and a wide range of different peatland sites as already noted by Crill et al. (1988) for CH_4 fluxes but also at different areas at the same peatland site.

The new peat quality index was sufficiently used to estimate the formation potential of both relevant greenhouse gases CO_2 and CH_4 from this fen. However, more research on different types of peatlands, i.e. *sphagnum*-peat bogs, boreal peatlands, Siberian bogs and degradated peatland sites is needed to confirm if this new quality index can be generally used to estimate the greenhouse potential of peat, i.e. for peatland restoration, permafrost development under changing climate conditions or rewetting and thawing events. Composition and microbial availability of C-based compounds are the most important factor controlling all microbial processes in peat soils, including anaerobic CO_2 and CH_4 formation as well as their atmospheric accumulation. Thus, the direct link presented here between peat quality and microbial activities will facilitate predictions that have a robust theoretical basis for modeling and calculating element cycles or trace gas fluxes from peatlands.

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GENERAL DISCUSSION

Cycling of iron

The fen Schlöppnerbrunnen is connected to a shallow groundwater layer and receives continuously Fe(II)-rich water from intermittent seeps and fens located upstream in the north-east of the Lehstenbach catchment area. There, Fe(II) is formed under waterlogged conditions [Küsel et al. 2008]. As shown in chapters I and II, Fe(III) accumulated near the peat surface of the fen due to peat oxygenation during drying, the mixing of oxic rainwater with reduced Fe(II) containing groundwater, and the radial loss of oxygen from growing vegetation. Taken together, these findings indicated that cycling of iron seem to be a significant process in the Lehstenbach catchment area (Figure III).

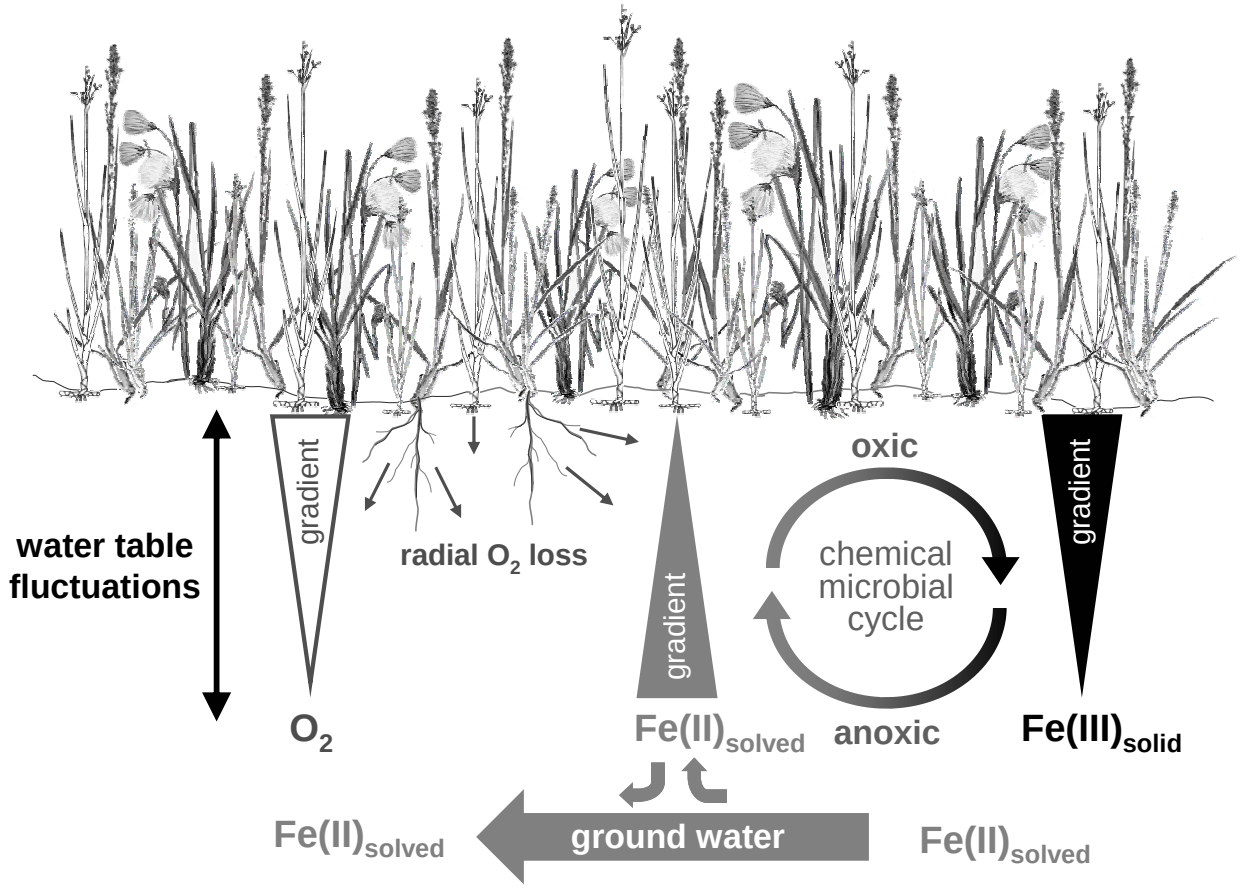

Figure III. Fe(II) oxidation and Fe(III) reduction involved in the iron cycle of a minerotrophic iron-rich fen influenced by peat oxygenation due to water table changes and growing vegetation.

Our experimental data highlighted the importance of Fe(II)-oxidizing prokaryotes (FeOP) which contribute substantially to the oxidation of Fe(II) in the Schlöppnerbrunnen fen. An associated diploma thesis completed by Claudia Lüdecke showed that FeOP increased Fe(II) oxidation rates by 1.5 times relative to chemical oxidation in sterile controls of Fe(II)-O_2 opposing gradient systems at pH ~ 5.5 (Figure IV). These gradient systems are favorable for the growth of microaerophilic chemolithoautotrophic FeOP [Emerson & Moyer 1997]. In contrast, FeOP do not alter the rate of Fe(III) oxide accumulation in circumneutral pH diffusion-controlled opposing-gradient culture systems [Sobolev & Roden 2004].

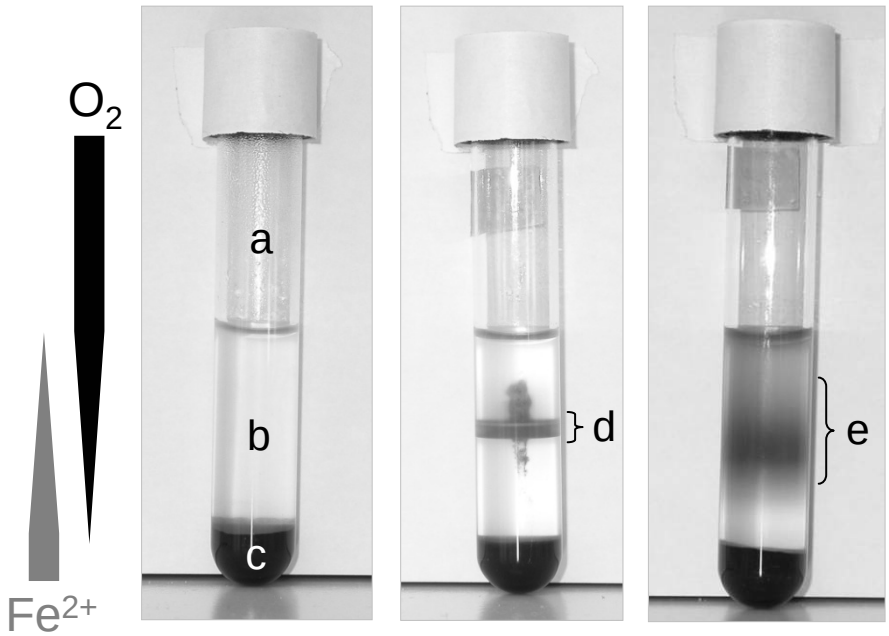

Figure IV: Fe(II)-O_2 opposing gradient systems used for cultivation of microaerophilic chemolithoautotrophic Fe(II)-oxidizing prokaryotes. The opposing gradient systems contained (a) a headspece of air with 21% oxygen; (b) Modified Wolfe`s Mineral Medium containing 0.2% agarose and flushed with CO_2 (pH ~ 5.5) as viscous overlayer; and (c) a solid FeS-plug containing 3% agarose as Fe(II) source (according to Emerson and Moyer [1997]). Overlayer of freshly prepared tubes was clear. In contrast, inoculated tubes with microbial Fe(II) oxidation resulted in the formation of a distinct rust colored band (d) located at the oxic/anoxic transition zone while chemical Fe(II) oxidation caused a diffuse rust colored cloud in sterile controls (e).

Scanning electron micrographs obtained from Fe(III) precipitates of the gradient systems suggest that microbially induced oxidation of Fe(II) yielded more fine-grained Fe(III) oxyhydroxides than chemically induced Fe(II) oxidation (Figure V). These amorphous Fe(III) oxide phases are high in reactive surfaces and thus, excellent substrates for FeRP [Emerson & Revsbech 1994, Roden & Zachara 1996].

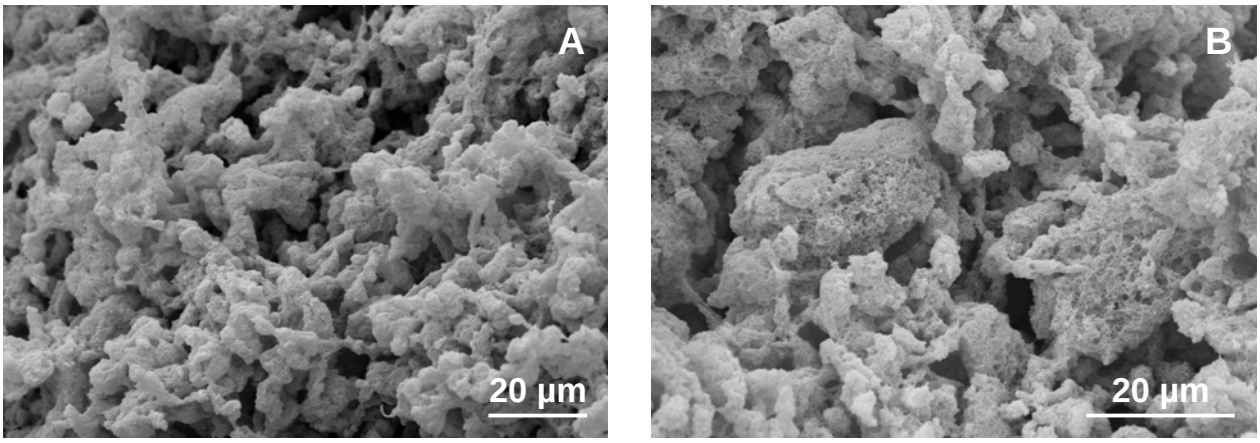

Figure V: Scanning electron micrographs of Fe(III) precipitates obtained from inoculated (A) and sterile (B) Fe(II)-O_2 opposing gradient systems. Samples were dried and coated with gold by a vacuum sputter.

Microaerophilic chemolithoautotrophic FeOP appeared to prefer gradients of Fe(II) and O_2 in the fen, as the highest abundances of micoaerophilic FeRP (up to 2.0 x 10^4 cells g (fresh wt peat)$^{-1}$) were observed in the 10-20 cm depth zone. In this zone, available oxygen and dissolved Fe(II) were generally present during the growing season and reached concentrations of 140 µM and 14%, respectively. Microelectrode measurements obtained in the gradient systems showed that microaerophilic FeOP were active up to a maximum oxygen saturation of 30%. At low pH, Fe(II) can be rapidly oxidized in acid mine drainage by *Acidithiobacillus ferrooxidans* and *Leptospirillum* spp. [Baker & Banfield 2003], whereas, in slightly acidic to neutral pH aquatic systems, *Gallionella ferruginea*, *Leptothrix* spp., and *Sphaerotilus natans* are involved in the oxidation of Fe(II) [Hallbeck & Pedersen 1990]. Analysis of enrichment cultures from the 10-20 cm depth zone of the fen revealed that 16S rRNA gene sequences were most closely related to the purple nonsulfur phototrophic organism *Rhodopseudomonas palustris,* to rhizosphere associated organisms like *Herbaspirillum* sp., *Janthinobacterium* sp. and *Bradyrhizobium japonicum,* and to *Acidobacterium capsulatum* an acidophilic, chemoorganotrophic bacterium (Figure VI). Using the gradient system technique, a pure culture was obtained that is closely related (98% sequence similarity) to *Siderooxidans lithoautotrophicus,* a circumneutral lithoautotrophic microaerophilic Fe(II) oxidizing bacterium isolated from Fe(II) rich groundwater [Emerson & Moyer 1997]. Anaerobic, nitrate-dependent and anaerobic phototrophic FeOP [Widdel et al. 1993, Straub & Buchholz-Cleven 1998] should play a minor role in the fen due to the low availability of nitrate and the absence of light.

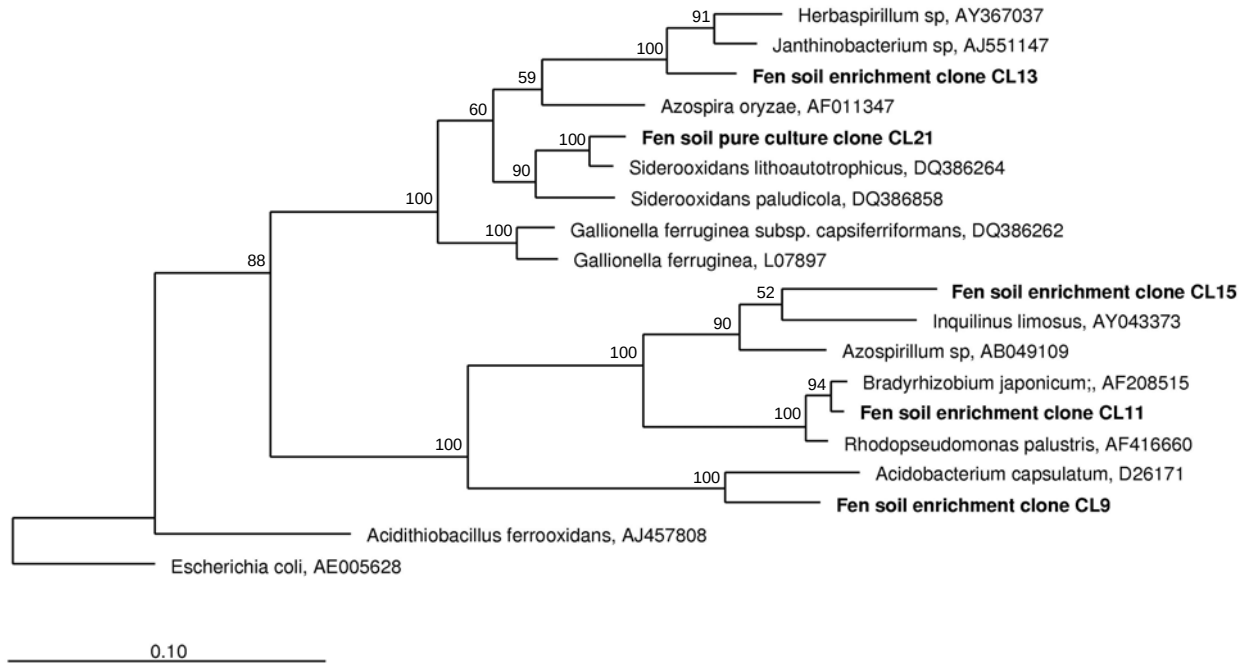

Figure VI: Phylogenetic tree based on 16S rRNA gene sequences showing the relative positions of enrichments and a pure culture obtained from Fe(II)-O_2 opposing gradient systems which favor the growth of chemolithoautotrophic microaerophilic Fe(II)-oxidizing prokaryoates as inferred by Parsimony method. Samples derived from the fen soil (10-20 cm) were collected in December 2007. Bootstrap values (in percent) for a total of 1000 replicates are shown at the nodes. Names and accession numbers for closest relatives 16S rRNA gene sequences are given. The bar indicates 10% sequence divergence.

By altering the spatial locus of bioavailable Fe(III) oxyhydroxide deposition in the redox transition zone, i.e., through the production of Fe(III)-binding ligands, FeOP appear to induce a rapid, microscale coupling of iron oxidation and reduction at aerobic-anaerobic interfaces [Emerson & Moyer 1997, Roden et al. 2004] (Figure III). In the iron-rich fen, the increased amount of reducible Fe(III) in the upper peat layer led to increased rates of potential Fe(III) reduction compared with lower depths and was therefore, of major significance for the oxidation of carbon in this fen.

Growing vegetation may also enhance the cycling of iron in the rhizosphere through the leakage of easily available carbon substrates such as organic acids, alcohols and sugars [Toal et al. 2000, Crow & Wieder 2005]. These compounds can fuel microbial metabolism as a carbon source and by enhancing the availability of Fe(III) by serving as an organic ligands. Organic ligands can help to maintain Fe(III) in a soluble form [Luther et al. 1992], which allows for direct contact with microbial cells in surrounding anoxic microzones [Lovley & Woodward 1996]. DOC in the porewater of the fen would further enhance Fe(III) reduction, because humic compounds can serve as electron shuttles between FeRP and surface-bound Fe(III) sterically inaccesible to microorganisms [Lovley et al. 2004]. Incubations with

supplemental Fe(III)-ligands and electron shuttle suggested that these dissolved humic substances in the peat pore water may contain compounds that can accelerate Fe(III) reduction. In addition, evidence indicates that members of the genera *Shewanella* and *Geothrix* are able to produce and release their own Fe(III)-ligands and electron shuttles [Nevin & Lovley 2002, Lovley et al. 2004].

Figure VII: Phylogenetic tree showing the relative positions of *Acidiphilium*-affiliated 16S rRNA gene sequences derived from 0-10 cm (zone I) and 30-40 cm (zone IV) depth of the fen soil obtained in June 2007 as inferred by Parsimony method. Bootstrap values (in percent) for a total of 1000 replicates are shown at the nodes. Names and accession numbers (brackets) for closest relatives 16S rRNA gene sequences are given. The bar indicates 10% sequence divergence.

Figure VIII: Phylogenetic tree showing the relative positions of *Geobacter*-affiliated 16S rRNA gene sequences derived from 0-10 cm (zone I) and 30-40 cm (zone IV) depth of the fen soil obtained in June 2007 as inferred by Parsimony method. Bootstrap values (in percent) for a total of 1000 replicates are shown at the nodes. Names and accession numbers (brackets) for all 16S rRNA gene sequences used for comparison are given. The bar indicates 10% sequence divergence.

PCR performed with 16S rRNA gene primers specific for known Fe(III)-reducing prokaryotes (FeRP) [Blöthe et al. 2008] yielded products for *Acidiphilium, Geobacter, Geothrix* and *Anaeromyxobacter* in all peat samples down to 40 cm depth. Surprisingly, no PCR products using *Acidithiobacillus* or *Shewanella* targeted primers were obtained from the fen, although microorganisms from these genera are common to many metal-reducing environments [Roden et al. 2004, Blöthe et al. 2008]. *Acidiphilium* affiliated clones of the upper 0-10 cm peat zone were most closely related to an uncultured forest soil clone, whereas, clones of deeper 30-40 cm peat zone were most closely related to an uncultured acidic *Sphagnum* peat bog clone (Figure VII). Heterotrophic acidophiles of the genus *Acidiphilium* appear to be widely distributed in metal-rich acidic environments [Küsel et al. 1999, Hallberg & Johnson 2003, Blöthe et al. 2008]. However, no clone sequence obtained from the fen matched to the cluster of cultivated *Acidiphilium* sp. sequences. *Geobacter* affiliated clones obtained from upper 0-10 cm peat zone were most closely related to cultured *Geobacter* sp., e.g., *Geobacter psychrophilus* or *Geobacter lovleyi*, whereas, clones obtained from deeper 30-40 cm peat zone were affiliated with cultured FeOP such as *Siderooxidans* sp. and *Gallionella* sp. (Figure VIII). *Geobacter* species are often the predominant organisms when extracellular electron transfer is an important process in subsurface environments. In addition, they can oxidize organic compounds completely to CO_2 with Fe(III) or Mn(IV) serving as the sole electron acceptor [Lovley et al. 2004]. *Geobacter* sp. have previously been classified as strict anaerobes, but there is evidence that they can tolerate and grow with oxygen as the sole electron acceptor at concentrations of up to 10% [Lin et al. 2004]. Thus, *Geobacter* sp. are likely involved in the reduction of Fe(III) in the iron rich and temporarily oxygenated upper peat zone.

The obtained results and observations of this thesis agreed with the first hypothesis that "Oxygenation of peat increase the extent of potential Fe(III) reduction and enhance the microbial cycling of iron.

Carbon mineralization influenced by changing redox conditions

a) Peat oxygenation and exoenzymatic activities

Two drying and rewetting field experiments were performed during the summer of 2006 and 2007. As natural weather conditions have a strong effect on the success of planned field experiments unexpected disturbances occur due to variations in mean temperatures and rainfall patterns. Thus, a prolonged dry period and a warm dry summer in 2006 caused the water table to be low and resulted in an extensive oxygenated zone at both the control and manipulation plots. However, we intensified these extremes by further lowering the groundwater level. In contrast, as a result of the cold and humid weather, drying was less efficient in 2007. As a consequence, only slight differences in peat oxygenation and water table level between control and manipulation plots occurred. During both manipulations the volumetric water content was still high and suggests that the high water holding capacity and capillary force of peat prevent from a rapid dehydration during the water table drawdown. Consequently, the increase of water table fluctuations as expected during future climate change may have less effect on the moisture content at this fen and may prevent a short-term flush in CO_2 emission as it has been shown in mineral soils after rewetting events [Kieft et al. 1987, Iovieno & Baath 2008]. However, increased pore volume during lowered water table increases the penetration of O_2 into the peat [Clymo 1983]. This oxygenation leads to a reoxidation of the pool of reduced compounds and a shift from anaerobic to more efficient aerobic mineralization. Our FeS redox probe data indicated that an increase in peat oxygenation did not always accompany a lowering of the water table, as was observed in other studies [Freeman et al. 1993a, Freeman et al. 1996]. The use of FeS redox probes is an efficient technique and useful tool for recording the maximum oxygen penetration depth during a defined exposure period at a field site.

Hydrolytic exoenzymatic activity was highest in the upper 10 cm of the fen and exoenzymatic activity in the surface peat layer increased during water table drawdown in 2007. Although additional phenolic compounds were able to decrease exoenzymatic activities, as shown in our peat slurry experiments and also by other authors [Vuorinen & Saharinen 1996, Freeman et al. 2001], no significant inhibition was found in field fresh peat during the manipulation period. In the fen, phenolics reached the highest (up to 8 mg L^{-1}) concentration between 10 and 20 cm depth. No activity of phenoloxidases was observed during peat oxygenation which could explain the absence of the phenolic compounds near the surface at the end of pumping [Freeman et al. 2001]. Alternatively, phenolics might be

eliminated from the soil solution by adsorption on freshly precipitated Fe(III) oxyhydroxides [Gu et al. 1994, Kalbitz et al. 2000] which would reduce their inhibiting effect on exoenzyme activity. The interaction of iron and phenolics may be supported by the concomitant increase in the concentrations of Fe(II) and phenolics in 10-20 cm depth at the end of pumping in 2007, due to Fe(III)-reduction processes.

Phosphatase activity was highest compared with ß-glucosidases and FDA-hydrolyzing enzymes. The high hydrolytic activities for phosphatases and the absence of dissolved phosphate in the soil water suggest that this fen is phosphate limited, despite the high amounts of total phosphorus (P) in the solid phase. Phosphate can be closely related to the cycling of iron [Zak et al. 2004] and the presence of its bioavailable form (dissolved orthophosphate, PO_4^{3-}) is dependent upon the redox conditions. Oxic conditions promote precipitation of PO_4^{3-} with Fe(III) oxyhydroxides and during Fe(III)-reduction it can redissolve. Thus, P cycling is vulnerable to changes in peatland hydrology [Reddy & Dangelo 1994]. However, porewater concentrations were below detection limits (< 1 µM) and sequential extractions of reduced and oxidized peat samples, according to Psenner et al. [1984], suggested that phosphate is not redox sensitive in this fen.

b) Formation of CO_2

Aerobic soil respiration rates were 1.4 times higher than anaerobic CO_2 formation rates during the water table drawdown of 2007 in the surface peat layer. In contrast, aerobic soil respiration rates have been proposed to be up to 4.3 times higher than anaerobic respiration rates [Bridgham & Richardson 1992, Moore & Dalva 1997, Bergman et al. 1999], and the CO_2 emission rate is three-fold higher at dry peatland locations than in water saturated peatlands [Jaatinen et al. 2008]. The uppermost peat horizon is located above the regular water level and is often oxygenated throughout the year. It is assumed that microbes in this layer are adapted to fluctuations in redox regimes and consequently, no clear differences between manipulation and control sites were found at that depth. Despite oxygenation of deeper peat (below 10 cm), aerobic and anaerobic CO_2 formation in these peat layers was not affected by the lowered water table in 2006 and 2007. In 2006, water table drawdown did not significantly effect CO_2 field emissions. Whereas, in 2007, significantly higher CO_2 emissions were observed at the control plots during two extraordinary dates with high air temperature and low air humidity (Muhr et al. unpublished). In addition, total C release during manipulations appeared to be less affected compared to other peatland studies [Jaatinen et al. 2008]. Thus, extreme water table drawdown caused by droughts did not further enhance CO_2

formation in this fen, as gaseous emissions were restricted to short periods and deeper oxygenated peat layers did not substantially contribute to the emission of CO_2.

c) Formation of CH_4

Oxygenation of reduced surface peat may lead to a rapid renewal of the Fe(III) pool which could result in the inhibition of methanogenic processes [Roden & Wetzel 1996] and a partial shift of the electron flow from CO_2 to Fe(III). This shift would explain the concomitant reduction processes observed in peatlands [Metje & Frenzel 2005, Dettling et al. 2006, Paul et al. 2006]. Overlapping Fe(III)-reducing and CH_4-forming activities were observed in several peat incubations, even when amorphous Fe(III)-oxyhydroxide was added to peat microcosms during the methanogenic phase. These concomitant Fe(III)-reducing and CH_4-forming activities suggest that microorganisms are utilizing non-competitive substrates. The abundance of glucose utilizing FeRP was up to two orders of magnitude higher than acetate- or ethanol-utilizing FeRP. This suggests that fermentative microorganisms contribute substantially to the reduction of Fe(III) in this fen. Fermentative microorganisms can utilize a broad range of electron donors and therefore, could avoid direct competition with H_2- or acetate-utilizing methanogens. The ability of methanogens to interact with extracellular quinones, humic acids, and poorly crystalline Fe(III) oxides has raised the possibility that methanogens contribute to Fe(III) and humic acid reduction and can alternatively explain the inhibition of methanogenesis in Fe(III)-rich ecosystems [Bond & Lovley 2002, van Bodegom et al. 2004].

Our data indicated that prolonged anoxic and reduced conditions, induced by high water table levels, were necessary for the establishment of *in situ* methanogenic activities. This positive correlation of water table depth on methanogenesis has generally been described in peatlands [Matthews & Fung 1987, Roulet et al. 1992b]. Our anoxic peat incubations showed a dominance of acetoclastical methanogenesis without seasonal or depth dependent changes and suggests that acetate is an important intermediate in this fen. However, concentrations of dissolved acetate were low or negligible down to 40 cm depth at the C5 control and D5 experimental plots, suggesting a rapid *in situ* turnover rate. Acetoclastic methanogenesis dominates in peatlands with higher plant communities, e.g., *Carex* sp. [Kelley et al. 1992, Galand et al. 2005, Rooney-Varga et al. 2007], such as our fen which is dominated by *Carex*, *Juncus*, *Molinia*, and *Eriophorum* species. In contrast, oligotrophic fens, ombrotrophic bogs, and *Sphagnum* sp. dominated peatlands are often hydrogenotrophic [Galand et al. 2005]. Vascular plant cover may increase in northern wetlands in response to

global warming [Weltzin et al. 2003, Walker et al. 2006] and cause a shift in methanogenic pathways toward increased acetotrophy and CH_4 formation [Hines et al. 2008]. Thus, this fen site is a good model for studying the impact of increasing extreme weather conditions, such as summer droughts and heavy rainfalls, on decomposition processes in peatlands. Despite these predictions, only few peat zones yielded high rates of methanogenesis and no net emissions of CH_4 to the atmosphere were observed during the manipulations in 2006 and 2007 (Knorr, K. H., Goldberg, S., unpublished data), indicating that CH_4 production is not a significant pathway of carbon flow in this fen.

With respect to the second hypothesis that "Oxygenation of peat leads to the activation of phenoloxidases and an increase in exoenzymatic activities and CO_2 formation rates", the main outcome of the thesis was that phenol oxidases played a negligible role in the degradation of phenolic compounds. This suggests that further drying and rewetting events would not strongly enhance mineralization processes in this fen, because the uppermost peat layer was already adjusted to drying and oxygenation, and oxygenated deeper peat layers do not substantially contribute to CO_2 emissions.

Link of peat quality on microbial activities

Peat mineralization processes, as determined by exoenzymatic activities and the formation of CO_2 and CH_4, were highly spatially variable at the fen site and depended not only on depth but also on the sampling area. Spatial heterogeneity and decreasing microbial activities with increasing depth has been observed in other peatlands [Nilsson & Bohlin 1993, Freeman et al. 1995, van den Pol-van Dasselaar & Oenema 1999]. This is likely due to redox conditions and the water saturation [Strack et al. 2004], as well as the microbial availability of organic carbon substrates [Bridgham & Richardson 1992]. Thus, although the total amount of organic carbon is high (up to 55%), microbial CO_2 formation and CH_4 could be substrate limited, especially in peat zones below 10 cm at plots C5 and D5. Our results suggest that poorly decomposed plant biomass, that is rich in carbohydrates and lignin, is a prerequisite for CO_2 and CH_4 development in an acidic fen. In addition, the type of vegetation may also influence the chemical composition and degradability of peat [Moore et al. 2007]; yielding varying amounts of precursors [Zeikus 1983, Bohlin et al. 1989] available for anaerobic CO_2 formation and methanogenesis.

In order to rapidly estimate the potential for CO_2 and CH_4 formation according to the chemical composition of C based compounds we developed a fast and simple peat quality index based on the thermal degradability of peat. Thus, we establish the ratio between the sum of labile and recalcitrant carbon compounds and the proportion of highly humidified inert C-based compounds ($PQI = POM_{labile} + POM_{recalcitrant} / POM_{inert}$) as a new peat quality index (PQI). In principle, the lower the value of the quality index, the higher the quantity of inert C-based compounds in the peat. Correspondingly, a peat with a high quality index showed higher concentrations of easily biodegradable C-based compounds.

In contrast to other more simple indexes dealing with C to N or lignin to N ratios [Taylor et al. 1989, Valentine et al. 1994, Moore et al. 2005], our PQI was sufficient to explain spatial heterogeneity in microbial formation of CO_2 and CH_4. Peat microorganisms were active in CO_2 and CH_4 formation when the proportion of the labile carbon fraction was greater than 35% and the corresponding PQI exceeded 1.35. Since water saturation controls peat degradation [Clymo 1984, Hogg et al. 1992, Belyea 1996], peat quality in the southern waterlogged sampling areas, e.g., sD4 and M, was higher than samples obtained from hydrological and redox unstable areas, e.g., C5 and D5. However, the long-term stability of the present peat quality index is uncertain with respect to potential CO_2 and CH_4 formation rates. The direct link between peat quality and microbial activity represents a robust basis for modeling and calculating element cycles or trace gas fluxes from peatlands. Therefore, estimations can be made for the potential contribution of peatlands to the pool of greenhouse gases during peatland restoration processes, permafrost development during changing climate conditions or rewetting and thawing events.

With respect to the third hypothesis "A high proportion of thermal labile carbon compounds in peat is responsible for high rates of potential CO_2 and CH_4 formation." the main outcome of the thesis was that an increased amount of thermal labile carbon compounds is favorable for microbial mineralization processes and a requisite for the formation of significant amounts of CO_2 and CH_4.

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SUMMARY

Northern peatlands cover only 3% of the terrestrial surface, but store approximately 30% of the global soil carbon stocks, due to high water saturation and low oxygen availability, which prevents organic matter mineralization. On the other hand, peatlands are a source of greenhouse gases, e.g., CO_2 and CH_4, and are now the focus of numerous environmental studies, as global climate change will affect their function as carbon sink and source. Climate change models predict an increase in the global mean temperature and a considerable change in precipitation patterns. An increase in the number of drying and rewetting events may stimulate soil respiration and enhance renewal of alternative electron acceptors available for the microbial oxidation of organic matter under anoxic conditions.

This thesis is part of the Research Unit FOR 562 "Dynamics of soil processes under extreme meteorological boundary conditions". For this research, peat samples were collected at the acidic fen Schlöppnerbrunnen in the northern Fichtelgebirge area of northern Bavaria (Germany). The main aim of this thesis was to investigate microbial mineralization processes influenced by water table changes and peat quality with respect to Fe(III) reduction in peat zones from 0 to 40 cm depth. Dialysis chambers and Rhizon samplers were used to characterize Fe(II), sulfate, nitrate, polyphenolic compounds and pH in porewater along a depth profile. In addition, we evaluated microbial activities in peat by measuring exoenzymatic activity and CO_2 formation rates under aerobic and anaerobic conditions. Methanogenic and Fe(III) reduction processes were also addressed and the activity and diversity of Fe(III)-reducing prokaryotes and microaerophilic Fe(II)-oxidizing prokaryotes was investigated. The chemical composition of carbon-based compounds present within peat was linked to the anaerobic formation CO_2 and CH_4 in order to develop a new peat quality index. The new peat quality index is fast and simple and can be easily used to estimate the greenhouse gas potential of peat. A change in the water table was experimentally initiated by roof construction and drainage followed by controlled rewetting with artificial rainwater, while non-manipulated sites served as control.

Rates of exoenzymatic enzyme activity, microbial respiration, Fe(III) reduction and methanogenesis indicated that microbial activity was the highest in the upper 10 cm layer of the fen soil. This upper peat horizon is located above the regular water level and is often oxygenated during the year. The addition of chelators, e.g., EDTA and NTA, and the electron shuttling compound AQDS to peat incubations had little to no effect on the formation of Fe(II). This was likely caused by the accumulation of a large pool of bioavailable Fe(III) in

the upper peat layer of the fen and by the high concentration of dissolved humic substances present in the porewater. Fe(II) formation rates were lower in depths below 10 cm compared with peat in the shallower layers, likely due to the decreased concentration of bioavailable Fe(III).

Despite the decrease in Fe(III) reduction with the increase of soil depth, the numbers of Fe(III)-reducing prokaryotes (FeRP) cultivated under pH 5.5 conditions were similar throughout the peat profile. The number of acetate-, ethanol-, or lactate utilizing FeRP, as determined using most probable number techniques, were approximately 10^5 to 10^6 cells g (fresh wt peat)$^{-1}$. Fermentative glucose-utilizing FeRP were the most abundant group (10^9 cells g [fresh wt peat]$^{-1}$; approximately 0.2% of the corresponding DAPI numbers) and may have dominated the reduction of Fe(III) in this fen. Amplification of 16S rRNA gene sequences specific for known FeRP yielded PCR products specific for *Acidiphilium*-, *Geobacter*-, and *Geothrix*-, but not for *Shewanella*- or *Anaeroromyxobacter*-related sequences in peat samples obtained from the 0-10 cm and 30-40 cm depth layers.

Microaerophilic Fe(II)-oxidizing prokaryotes (FeOP) were in the highest abundance in the 10-20 cm depth layer (10^4 cells g (fresh wt peat)$^{-1}$), compared to 10^3 and 10^2 cells g (fresh wt peat)$^{-1}$ in 0-10 cm and 30-40 cm depth layers, respectively. In the 10-20 cm depth layer, temporarily oxic conditions and high concentrations of dissolved Fe(II) occurred. Enrichment cultures of microaerophilic FeOP obtained from Fe(II)-O_2 opposing gradient systems were most closely related to *Rhodopseudomonas* sp., *Acidobacterium* sp., *Gallionella* sp., and *Siderooxidans lithoautotrophicus* based on 16S rRNA gene sequences similarity. Microaerophilic FeOP increased Fe(II) oxidation rates by 1.5 times and microbial oxidation of Fe(II) yielded more fine-grained Fe(III) oxyhydroxides relative to chemical oxidation in sterile controls. Thus, FeOP appear to compete successfully with the chemical oxidation at oxic-anoxic interfaces in this fen, providing an easily available Fe(III) source for microbial Fe(III) reduction.

Methanogenesis typically began when Fe(II) formation reached a plateau. However, the ability of methanogens to use Fe(III) as electron acceptor and low substrate competition with FeRP allowed for concomitant electron accepting processes. High proportion of acetoclastical methanogenesis (~60%) indicated that acetate might be the main precursor for the formation of methane in this fen. However, total formation of CH_4 was highly spatially variable and dependent on the depth and sampling area.

Spatial heterogeneity of CO_2 and CH_4 formation rates could be explained by a newly developed peat quality index based on the thermal degradability of peat. This peat quality

index has been established as the ratio between the sum of labile and recalcitrant carbon compounds to the proportion of highly humified carbon compounds. In principle, a peat with a high quality index showed higher concentrations of easily biodegradable carbon and could be found mostly in the upper peat zone. Thus, the poorly decomposed plant biomass present in upper peat layers that is rich in carbohydrates and lignin seemed to be a prerequisite for CO_2 and CH_4 development from acidic fens.

During the water table manipulations the volumetric water content of peat remained high and differed little between manipulation and control sites. The high water holding capacity of peat likely prevented rapid dehydration while the water table was lowered and rewetting did not lead to a CO_2 flush. FeS redox probes demonstrated that oxygen penetration into the peat roughly followed the water table level. Under oxygenated conditions, rates of exoenzymatic activities and CO_2-respiration were enhanced in upper peat (0-10 cm depth) and although oxygenation reached deeper peat zones, activity was still low. The inhibitory effect of phenolic compounds on exoenzymatic activities was low and may explain the absence of phenol oxidases. In general, water table drawdown yielded a higher availability of nitrate, Fe(III), and sulfate and prolonged the onset of methane formation. Potential Fe(II) formation was increased in upper peat during lowered water table, apparently due to accumulation of bioavailable Fe(III).

In general, the increase of extreme weather conditions, such as frequent summer droughts and heavy rainfall events, which are predicted for the next decades may not enhance mineralization processes in this fen. Microorganisms in the upper, most active peat layer appeared to be adjusted to oxygenation, and deeper peat layers did not substantially contribute to CO_2 emissions. These findings strengthen the idea that peatlands function as a sink for nitrate and iron and as a source for sulfate. In addition, Fe(III) and sulfate will be favored as alternative electron acceptors due to the storage and enhanced re-oxidation of their reduced compounds in the peat.

ZUSAMMENFASSUNG

Obwohl Moore der nördlichen Hemisphäre nur 3% der Landoberfläche bedecken, speichern sie ca. 30% des gesamten globalen Kohlenstoffs. Ein hoher Wasserstand und die geringe Verfügbarkeit von Sauerstoff verringert die Mineralisation der organischen Substanzen und dennoch sind Moore als Quellen von klimarelevanten Gasen wie Methan und Kohlendioxid bekannt. Klimamodelle prognostizieren nicht nur einen Anstieg der globalen Durchschnitts-temperaturen sondern auch eine Veränderung von lokalen Niederschlagsereignissen. Da diese Veränderungen einen Einfluss auf die Kohlenstoffdynamik in Mooren erwarten lassen, stehen mikrobielle Prozesse und Nährstoffkreisläufe im Fokus von einer Vielzahl von wissen-schaftlichen Untersuchungen. Ein Anstieg von Trocken- und Wiedervernässungs-ereignissen sollte demnach nicht nur die mikrobielle CO_2-Respiration erhöhen, sondern auch zu einer Erneuerung und einem Anstieg des Pools von alternativen Elektronenakzeptoren führen, welcher dann für den mikrobiellen Abbau von organischem Material zur Verfügung stehen.
Diese Promotionsarbeit wurde im Rahmen der Forschergruppe 562 „Einfluss auf Boden-prozesse bei extremen meteorologischen Randbedingungen“ angefertigt und Torfproben im Schlöppnerbrunnen Moor im Lehstenbach Einzugsgebiet im Norden Bayerns (Deutschland) entnommen. Das Ziel dieser Arbeit bestand darin, den Einfluss von wechselnden Wasserständen und unterschiedlichen Torfqualitäten auf die mikrobielle Reduktion von Fe(III) und anderer mikrobielle Mineralisationsprozesse in Torf bis zu einer Tiefe von 40 cm zu untersuchen. Saugkerzen und Dialysesammler wurden benutzt, um chemische Parameter wie Fe(II), Sulfat, Nitrat, polyphenolische Substanzen und den pH über die Tiefe zu bestimmen. Exoenzymatische Aktivitäten sowie aerobe und anaerobe Respirationsraten wurden ermittelt, um die allgemeine mikrobielle Aktivität im Torf bestimmen zu können. Des Weiteren wurden methanogene und Fe(III)-reduzierende Prozesse unersucht und die Aktivität und Diversität von Fe(III)-reduzierenden und Fe(II)-oxidierenden Mikroorganismen ermittelt. Ein schneller und einfacher Torfqualitätsindex wurde erstellt, um das Treibhausgaspotential des Torfes widerspiegeln zu können. Dazu wurde die chemische Zusammensetzung der Kohlenstoff-verbindungen in Torfproben mit der anaeroben Bildung von CO_2 und CH_4 verglichen. Wasserstandsmanipulationen konnten im Moor gezielt durch das Errichten von Dächern und dem Einbringen von Drainagen kontrolliert und die Wiedervernässung später durch Regenwassersurugat initiiert werden. Unmanipulierte Flächen dienten als Kontrollen.
Im Allgemeinen waren die Raten für die mikrobielle Respiration, exoenzymatischen Aktivitäten, Fe(III)-Reduktion und Methanbildung in den oberen 10 cm des Torfes am

höchsten. Diese oberste Torfschicht befindet sich normalerweise über dem Grundwasserspiegel und wird daher von wechselnden Redoxbedingungen beeinflusst. Die Zugabe von Chelatbildnern wie EDTA und NTA sowie dem Elektronenshuttle AQDS hatte keinen oder nur einen geringen stimulierenden Effekt auf die Bildung von Fe(II). Dies war auf die Anreicherung von mikrobiell leicht reduzierbarem Fe(III) in der oberen Torfschicht und auf die hohe Konzentration von Huminstoffen im Moorporenwasser zurückzuführen. Aufgrund geringerer Konzentrationen von mikrobiell verfügbarem Fe(III) waren die Fe(II) Bildungsraten in Tiefen unterhalb von 10 cm gering.

Obwohl eine Abnahme der Fe(III)-Reduktionsraten mit ansteigender Tiefe beobachtet werden konnte, waren die Abundanzen der bei pH 5,5 kultivierten Fe(III)-Reduzierer (FeRP) über die Tiefe nahezu konstant. Die Abundanzen der acetat- ethanol- und laktatverwertenden FeRP erreichten in MPN (Most probable number)-Versuchen 10^5 bis 10^6 Zellen g (Frischmasse Torf)$^{-1}$, wohingegen gärende FeRP die abundanteste Gruppe (10^9 Zellen g [Frischmasse Torf]$^{-1}$, entspricht ca. 0,2 % der Gesamtzellzahl im Torf) darstellte. Im Gegensatz zu *Shewanella*- und *Anaeroromyxobacter*-verwandten Sequenzen konnten spezifische PCR-Produkte für *Acidiphilium*-, *Geobacter*-, und *Geothrix*-verwandte Sequenzen in Torfproben von 0-40 cm nachgewiesen werden.

Verglichen mit 10^3 und 10^2 Zellen g (Frischmasse Torf)$^{-1}$ in den Tiefen 0-10 cm und 30-40 cm, erreichten mikroaerophile Fe(II)-Oxidierer (FeOP) mit 10^4 Zellen g (Frischmasse)$^{-1}$ die höchste Abundanz in einer Tiefe von 10-20 cm. Dort konnte neben temporär auftretenden oxischen Bedingungen auch hohe Fe(II) Konzentrationen nachgewiesen werden. *Rhodopseudomonas*-, *Acidobacterium*-, *Gallionella*- und *Siderooxidans lithoautotrophicus* – verqwandte 16S rRNA-Sequenzen konnten in Anreicherungskulturen von mikroaerophilen Fe(II)-Oxidierern nachgewiesen werden. Die Fe(II) Oxidationsraten konnten im Vergleich zur chemischen Oxidation in sterilen Kontrollen durch das Vorhandensein von FeOP um das 1,5-fache erhöht werden und entstandene Fe(III)-oxide wiesen dabei eine feinkörnigere Struktur auf. FeOP können somit nicht nur mit der chemischen Oxidation an oxisch-anoxschen Grenzflächen im Moor konkurrieren, sondern auch mikrobiell leicht reduzierbares Fe(III) zur Verfügung stellen.

Die Bildung von Methan setzte normalerweise erst ein, wenn die Bildung Fe(II) die Plateauphase erreicht hatte. Dennoch konnten in einigen Fällen überlappende Aktivitäten beobachtet werden, die auf eine geringe Konkurrenz um vorhandene Substrate sowie die Möglichkeit einiger Methanogener Fe(III) als Elektronenakzeptor zu nutzen zurückzuführen sind. Da der acetoklastische Anteil an der Methanbildung rund 60 % betrug, scheint Acetat

ein wichtiges Substrat für die Bildung von CH_4 in diesem Moor zu sein. Generell war die Bildung von CH_4 vom Probenahmestandort und der jeweiligen Torfschicht abhängig.

Räumliche Unterschiede der CO_2- und CH_4-Bildungsraten konnten generell durch einen neu entwickelten Torfqualitätsindex als Verhältnis zwischen der Summe der thermisch labilen und weniger labilen Kohlenstoffverbindungen mit dem Anteil von stark humifizierten Kohlenstoffverbindungen erklärt werden. Demnach wiesen vor allem Torfproben nahe der Oberfläche einen hohen Qualitätsindex und einen erhöhten Anteil von mikrobiell leicht abbaubarem organischem Material auf. Dieser Torf bestand im wesendlichen aus gering zersetzter, kohlenhydrat- und ligninhaltiger pflanzlicher Biomasse, welche eine wichtige Voraussetzung für die Bildung von CO_2 und CH_4 zu sein schien.

Während der Austrocknungsexperimente blieb der Wassergehalt des Torfes nahezu unverändert. Es ist daher anzunehmen, dass die hohe Wasserhaltekapazität des Torfes eine schnelle Austrocknung während schwankender Wasserstände verhindert. Eine starke CO_2 Emission nach Wiedervernässung, wie sie in terrestrischen Böden beobachtet werden kann, konnte daher nicht beobachtet werden. FeS-Redox-Stäbe zeigten, dass die Eindringtiefe von Sauerstoff in den Torfkörper weitestgehend dem Grundwasserstand folgte. Obwohl oxische Bedingungen während der Wasserstandsabsenkungen zu einer Erhöhung der CO_2-Respiration und exoenzymatischen Aktivitäten in der oberen Torfschicht führten, blieben diese Raten in darunterliegenden Tiefen nahezu unverändert. Ein Anstieg der Phenoloxidaseaktivitäten konnte unter oxischen Bedingungen nicht nachgewiesen werden und generell schien der hemmende Einfluss von phenolischen Verbindungen auf die Aktivität der Exoenzyme im Moor gering zu sein. Die Wasserstandsabsenkungen führten weiterhin zu einem Anstieg der Nitrat-, Fe(III)- und Sulfatkonzentrationen, wohingegen die Bildung von CH_4 verzögert wurde. Die Akkumulation von mikrobiell leicht verfügbarem Fe(III) in der oberen Torfschicht erhöhte die potentiellen Fe(II)-Bildungsraten.

Die erwartete Zunahme von extremen Wetterereignissen, wie einer Häufung von Sommerdürren und Starkregenereignissen, scheint in diesem Moortyp zu keiner verstärkten Mineralisierung der organischen Substanz zu führen, da die Mikroorganismen in der mikrobiell aktivsten oberen Torfschicht an oxische Bedingungen angepasst sein sollten und die tieferen Schichten generell zu keiner wesentlichen CO_2-Emissionen beigetragen haben. Dennoch, die Anreicherung von Nitrat, Fe(III) und Sulfat im Torfkörper, durch eine Zunahme von wechselnden Redoxbedingungen sollte in einer verstärkten mikrobiellen Nutzung dieser alternativen Elektronenakzeptoren resultieren.

APPENDIX

MICROBIAL REDUCTION OF IRON AND POREWATER BIOGEOCHEMISTRY IN ACIDIC PEATLANDS

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Abstract

Temporal drying of upper soil layers of acidic methanogenic peatlands might divert the flow of reductants from CH4 formation to other electron-accepting processes due to a renewal of alternative electron acceptors. In this study, we evaluated the in situ relevance of Fe(III)-reducing microbial activities in peatlands of a forested catchment that differed in their hydrology. Intermittent seeps reduced sequentially nitrate, Fe(III), and sulfate during periods of water saturation. Due to the acidic soil conditions, released Fe(II) was transported with the groundwater flow and accumulated as Fe(III) in upper soil layers of a lowland fen apparently due to oxidation. Microbial Fe(III) reduction in the upper soil layer accounted for 26.7 and 71.6% of the anaerobic organic carbon mineralization in the intermittent seep and the lowland fen, respectively. In an upland fen not receiving exogenous Fe, Fe(III) reduction contributed only to 6.7%. Fe(II) and acetate accumulated in deeper porewater of the lowland fen with maximum concentrations of 7 and 3 mM, respectively. Both supplemental glucose and acetate stimulated the reduction of Fe(III) indicating that fermentative, incomplete, and complete oxidizers were involved in Fe(II) formation in the acidic fen. Amplification of DNA yielded PCR products specific for *Acidiphilium*-, *Geobacter*-, and *Geothrix*-, but not for *Shewanella*- or *Anaeroromyxobacter*-related sequences. Porewater biogeochemistry observed during a 3-year-period suggests that increased drought periods and subsequent intensive rainfalls due to global climate change will further favor Fe(III) and sulfate as alternative electron acceptors due to the storage and enhanced re-oxidation of their reduced compounds in the soil.

Keywords: porewater biogeochemistry, *Acidiphilium*, *Geobacter*, iron reduction, peatland

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Microbial reduction of iron and porewater biogeochemistry in acidic peatlands

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Abstract. Temporal drying of upper soil layers of acidic methanogenic peatlands might divert the flow of reductants from CH_4 formation to other electron-accepting processes due to a renewal of alternative electron acceptors. In this study, we evaluated the in situ relevance of Fe(III)-reducing microbial activities in peatlands of a forested catchment that differed in their hydrology. Intermittent seeps reduced sequentially nitrate, Fe(III), and sulfate during periods of water saturation. Due to the acidic soil conditions, released Fe(II) was transported with the groundwater flow and accumulated as Fe(III) in upper soil layers of a lowland fen apparently due to oxidation. Microbial Fe(III) reduction in the upper soil layer accounted for 26.7 and 71.6% of the anaerobic organic carbon mineralization in the intermittent seep and the lowland fen, respectively. In an upland fen not receiving exogenous Fe, Fe(III) reduction contributed only to 6.7%. Fe(II) and acetate accumulated in deeper porewater of the lowland fen with maximum concentrations of 7 and 3 mM, respectively. Both supplemental glucose and acetate stimulated the reduction of Fe(III) indicating that fermentative, incomplete, and complete oxidizers were involved in Fe(II) formation in the acidic fen. Amplification of DNA yielded PCR products specific for *Acidiphilium*-, *Geobacter*-, and *Geothrix*-, but not for *Shewanella*- or *Anaeroromyxobacter*-related sequences. Porewater biogeochemistry observed during a 3-year-period suggests that increased drought periods and subsequent intensive rainfalls due to global climate change will further favor Fe(III) and sulfate as alternative electron acceptors due to the storage and enhanced re-oxidation of their reduced compounds in the soil.

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1 Introduction

Acidic wetlands (pH<5.0) represent a vast type of northern wetlands in Eurasia and North America (Harriss et al., 1993). Waterlogging, low temperatures, and low nutrient quality of plant litter impair decomposition of plant litter, favoring the accumulation of organic carbon. Emission estimates of the greenhouse gas methane (CH_4) from wetlands range from 92 to 232 Tg CH_4 $year^{-1}$ (Wuebbles and Hayhoe, 2002). Although rates of CH_4 production were shown to be correlated with water-table depth, peat chemistry and vegetation type (Verville et al., 1998), pathways of CH_4 production are still not well understood. Generally, two-thirds of the biogenic CH_4 produced in wetlands originates from acetoclastic methanogenesis (Conrad, 1999). However, H_2-CO_2 appears to be a significant precursor in northern peatlands (Avery et al., 2003; Horn et al., 2003). Acetate even accumulates in some peatlands as a terminal product of anaerobic decomposition indicating that it is not the primary source of CH_4 that is emitted from such habitats (Hines et al., 2001; Duddleston et al., 2002). Acetate consumption appears to occur in these peatlands after diffusion into oxic environments where it is oxidized to carbon dioxide (CO_2).

High-latitude regions are expected to experience a temperature increase as a result of global climate change, and climate models predict a decrease in annual precipitation in most European regions during the next decades (International Panel on Climate Change (IPCC), 2007). Thus, transient drying and oxidation of upper soil layers might divert the flow of reductants from CH_4 formation (Blodau et al., 2004) to other electron-accepting processes due to the renewal of alternative electron acceptors. Atmospheric nitrogen and sulfate depositions might further enhance the activity of sulfate and nitrate reducers under changed climatic conditions in northern peatlands similar to other wetlands (Gauci et al., 2002; Lamers et al., 2002; Vile et al., 2003). However, addition of alternative electron acceptors to ombrotrophic bogs and minerotrophic

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Table 1. Characteristics of the seep and both fens from a forested catchment[a].

Field site	Vegetation	Groundwater tabledepth (m)	Soil type	Depth (cm)	Dry weight (%)	pH ($CaCl_2$)	C_{org} (%)	N_{total} %	Fe_d ($g\,kg^{-1}$)[b]	Fe_o ($g\,kg^{-1}$)[c]
Intermittent seep	*Sphagnum* mosses, *Vaccinium myrtilus*	0.1-to-1.0	Dystric Gleysol	0–10	17.8	3.2	37.2	2.0	2.1	2.0
				10–20	19.5	3.4	42.9	2.4	3.6	3.0
				20–30	23.5	3.6	34.7	1.9	2.7	2.3
upland fen	*Sphagnum* mosses, *Carex* sp., some spruce stockings	0.2	Fibric Histosol	0–10	10.6	4.1	42.4	1.8	0.5	0.5
				10–20	9.5	4.2	44.0	1.9	0.4	0.4
				20–30	9.6	4.4	39.4	2.1	0.3	0.4
lowland fen	*Molinia caerulea*, *Eriophorum vaginatum*	0.1	Fibric Histosol	0–10	5.7	4.6	38.0	1.9	18.9	14.2
				10–20	10.0	4.3	40.0	1.5	9.8	8.7
				20–30	13.6	4.4	34.3	1.6	3.4	2.7

[a] Presented are the averages from duplicate soil samples.
[b] Fe_d refers to pedogenic Fe-oxides.
[c] Fe_o refers to poorly crystallized Fe-oxides, hydroxides, and associated gels.

fens incubated in anoxic jars do not universally divert carbon and electron flow from CH_4 formation (Dettling et al., 2006).

$\delta^{34}S$ values and ^{35}S-labeling patterns indicate that the dissimilatory reduction of sulfate is an ongoing process in acidic seeps and fens of a forested catchment in northern Bavaria, Germany (Lehstenbach, Fichtelgebirge) (Alewell and Giesemann, 1996; Alewell and Novak, 2001) like in peat bogs (Wieder and Lang, 1988). In minerotrophic fens which are connected to the groundwater flow, ferric iron [Fe(III)] can be another potential electron acceptor. Fe(II) concentration profiles hint to microbial Fe(III) reduction in pH neutral fens adjacent to agricultural fields (Todorova et al., 2005), and Fe(III) reduction appears to parallel CH_4 formation in northern acidic wetlands (Metje and Frenzel, 2005). Fe(III) reduction might compete with methanogenesis for H_2 or acetate (Roden and Wetzel, 2003). Most Fe(III) reducers can use one or more alternative electron acceptors (Lovley et al., 2004), which might be advantageous in upper peat layers that experience redox fluctuations due to water-table variations or oxygen release by plant roots. While Fe(III) exists predominantly in the solid phase as oxyhydroxide minerals at circumneutral pH, Fe(III) is more soluble under acidic conditions (Lovley et al., 2004). However, most cultured Fe(III) reducers are neutrophiles and have only negligible capacities to reduce Fe(III) under moderately acidic (pH 3-6) conditions. Thus, we have only a marginal understanding of the flow of carbon and reductant in acidic, Fe(III) rich habitats and of their inherent Fe(III) reducing microbial communities (Vile and Wieder, 1993; Cummings et al., 2000, 2003; Blodau et al., 2002; Petrie et al., 2003; Adams et al., 2007; Blöthe et al., 2008).

The main objectives of this study were (1) to provide experimental evidence on field based data for diverted flow of reductants from CH_4 formation to other electron accepting processes in upper soil layers of peatlands which differ in their hydrology, and (2) to achieve a better understanding of the flow of carbon in acidic habitats and of their inherent Fe(III)-reducing community.

2 Material and methods

2.1 Field site description

The sites are located in the Lehstenbach catchment (Fichtelgebirge, Northeastern Bavaria, Germany), with a highest elevation of 877 m a.s.l. and an area of 4.2 km^2. Ninety % is covered by Norway spruce (*Picea abies* [L.] KARST.) of different age classes, and thirty % of the catchment soils are fens and seeps. Upland soils in the catchment have developed from weathered granitic bedrock and are predominantly Dystric Cambisols and Cambic Podzols (WRB system). The annual precipitation in the catchment varies between 900 and 1160 mm yr^{-1} and the average annual temperature is 5°C. The site Schlöppnerbrunnen I (50°08′14″ N, 11°53′07″ E) is a fen which is located in the upper part of the catchment (upland fen), dominated by *Sphagnum* mosses alternately covered with patches of *Vaccinium myrtillus* (L.), *Juncus effusus* (L.), *Carex nigra* ((L.) Reichard)), *Carex rostrata* (Stokes), and *Carex canescens* (L.) (Table 1). The yearly mean groundwater table depth approximated 0.2 m. Some lower situated soils in the catchment Lehstenbach are only water saturated if the groundwater level increases during autumn and winter. In these seeps, represented by the site Köhlerloh (intermittent seep), the groundwater level reaches the soil surface during autumn, winter, and spring, whereas it is about 0.5-to-1 m below the surface during summer. Open areas at the seep are vegetated with *Sphagnum* mosses or are partly covered with dense layer of *Vaccinium myrtilus*. The fen Schlöppnerbrunnen II (50°08′38″ N, 11°51′41″ E) is completely overgrown by *Mollinia caerulea (L. Moench)*,

Eriophorum vaginatum (L.), *Carex canescens* (L.), and *Juncus effusus* (L.). It is located down slope in the catchment and close to the runoff (lowland fen). The mean groundwater table approximated 0.1 m.

Three intermittent seeps located upstream of the lowland fen were also sampled in October 2003. These seeps were covered with spruce, *Vaccinium myrtillus*, and some *Sphagnum* mosses.

2.2 Porewater collection

Porewater from the upper 40 cm of the fens site was sampled with dialysis chambers every two months during the time period from the end of July 2001 to July 2004 with an interruption during the winter months due to coverage of the catchment with snow and ice as previously reported (Schmalenberger et al., 2007). The intermittent seep was only sampled during periods of water saturation. The hot summer of 2003 lead to a drying of the topsoil of the lowland and the upland fen down to a depth of 5-to-10 cm.

2.3 Anoxic soil microcosms

For determining rates of anaerobic microbial activities, soil samples from three replicate sites and different depths (approximately 0–10, 10–20, and 20–30 cm) were obtained in September 2001 in sterile airtight vessels and transported to the laboratory. Replicates were pooled and processed within 4 h. Fourty g (wet wt) soil was placed into 125-ml infusion flasks (Merck ABS, Dietikon, Switzerland) inside an O_2-free chamber (100% N_2 gas phase). Bottles were closed with rubber stoppers and screw-cap seals, flushed with sterile argon for 15 min, and incubated in the dark at 15°C with an initial overpressure of 20–25 kPa argon at room temperature. Headspace gases were taken by sterile, argon flushed syringes from these bottles. Gas values were estimated by Henry's law and included the total amounts in both gas and liquid phases. To facilitate sampling of water soluble parameters, 40 ml anoxic, deionized water with a pH of 5.0 were added to another set of soil microcosms with 40 g (wet wt) soil. Adjustments of pH were performed with sterile solutions of 10 N HCl and 10 N NaOH. All microcosms were done in three replicates. At 8 time points, samples were taken during 15 days of incubation in the dark at 15°C. Activity rates were calculated by linear regression analysis during the time period of linear increase of reduced compounds or linear decrease of electron acceptors.

For determining the effect of supplemental electron donors on Fe(III)-reducing activities, soil samples (0–10 cm depth) were obtained from the lowland fen in March and October 2002. Thirtyfive g (wet wt) soil was mixed with 70 ml anoxic, deionized water with a pH of 5.0. Glucose (2.5 mM), acetate (2 mM), or lactate (2 mM) was added from sterile anoxic stock solutions; H_2 (10 ml) was added as sterile gas. At 10 time points, samples were taken during 15 days of incubation in the dark at 15°C. Fe(II) formation rates were calculated by linear regression analysis during the time period of linear increase of Fe(II) (glucose, 4 days; acetate, 8 days; lactate, H_2 and control 7 days).

2.4 Enrichments of Fe(III) reducers

Five g (wet wt) soil of the lowland fen (0–10 cm) obtained in October 2003 was mixed with 95 ml of dilution buffer (Küsel et al., 2001) and further diluted in a tenfold dilution series, which were used to inoculate an anoxic, undefined medium with a pH of 5.2 that was supplemented with 40 mM amorphous ferric hydroxide [$Fe(OH)_3$] and either acetate (5 mM) or H_2 (10 ml) as previously described (Blöthe et al., 2008). Bromoethanesulfonate (BESA) was added (15 mM) to inhibit methanogenesis. Tubes were incubated in the dark at 15°C for 3 months.

2.5 Analytical methods

The pH was measured with a U457-S7/110 combination pH electrode (Ingold, Steinbach, Germany). Headspace gases (H_2, CO_2 and CH_4) were measured with Hewlett Packard Co. (Palo Alto, CA, USA) 5890 series II gas chromatographs (Küsel and Drake, 1995). The reduction of Fe(III) was determined after acid extraction by the amount of Fe(II) formed in anoxic incubations. Aliquots (0.2 ml) of the media or of the soil suspension were taken by sterile syringes and transferred to 9.8 ml of 0.5 N HCl and incubated for 1 h at room temperature (Küsel et al., 1999). Fe(II) was measured after the addition of acetate by the phenanthroline method (Tamura et al., 1974). Pedogenic iron (Fe_d) was extracted with dithionite-citrate-bicarbonate solution (Mehra and Jackson, 1960). Poorly crystallized iron oxides, hydroxides, and associated gels (Fe_o) were extracted with acidic ammonium oxalate solution (Schwertmann, 1964). Extracted Fe was measured by atomic absorption spectrometry (Unicam 939 spectrometer, ThermoNicolet GmbH, Offenbach, Germany). Short chain aliphatic acids and alcohols were determined with Hewlett-Packard 1090 series II high-performance liquid chromatographs (Küsel and Drake, 1995). The detection limits for short chain fatty acids approximated 5–10 μM and of alcohols 30 μM at an injection volume of 200 μl. Dissolved organic carbon (DOC) was analyzed with a liquiTOC (Foss-Heraeus, Hanau, Germany) with a detection limit of 50 μM. NH_4^+ and NO_3^-/NO_2^- were determined by flow injection analysis with a detection limit of 3 μM (QuickChemAE, Lachat Instruments, Milwaukee, WI). SO_4^{2-} was measured by ion chromatography with a detection limit of 3 μM (DX-100 with AS4 A column; Dionex, Sunnyvale, CA). Sulfide was measured by the Cline procedure with a detection limit of 5 μM (Cline, 1969).

Table 2. Anaerobic activities of seep and fen soils in anoxic microcosms of September 2001.

Site	Depth cm	Rate of formation or consumption [nmol g (wet wt soil)$^{-1}$ d^{-1}][a]					$Fe(II)^{b}_{max}$ [μmol g (wet wt soil)$^{-1}$]	Onset of CH_4 formation (days)
		NO_3^-	Fe(II)	SO_4^{2-}	CO_2	CH_4		
intermittent seep	0–10	−98.6	693	−21.0	649	1.6	8.1	11
	10–20	−48.0	487	1.2	215	0	5.5	n.a.[c]
	20–30	n.a.	544	4.8	198	0	3.5	n.a.
upland fen	0–10	−50.0	47	−19.0	175	25	0.7	1
	10–20	n.a.	19	−1.1	121	63	0.4	4
	20–30	n.a.	38	−0.2	44	30	0.4	4
lowland fen	0–10	n.a.	1177	−3.2	411	619	15.8	0
	10–20	n.a.	430	−0.34	253	244	8.5	0
	20–30	n.a.	379	−0.0	161	102	5.4	0

[a] Presented is the average rate observed in triplicate microcosms.
[b] Final Fe(II) concentrations after Fe(III)-reduction was completed.
[c] n.a. not applicable. No CH_4 was formed or no NO_3^- was present

2.6 DNA extraction, PCR amplification of 16S rRNA genes

DNA was extracted from lowland fen soil (0–10 cm depth) obtained in October 2003 and May 2007 using the MOBIO Power Soil DNA extraction kit according to manufacturer's instructions. Aliquots of DNA were PCR amplified using *Bacteria* domain-specific (GM3, GM4; Muyzer et al., 1995) and 16S rRNA gene primers specific for *Acidiphilium* (Acido594F, Acido1150R; Wulf-Durand et al., 1997), bioleaching-associated bacteria (Ferro458F/Ferro1473R; Wulf-Durand et al., 1997), *Geobacter* (GM3, 825R; Snoeyenbos-West et al. 2000), *Geothrix* (Gx182F, Gx472R; Snoeyenbos-West et al., 2000), *Shewanella* (Shw783F, Shw1245R; Snoeyenbos-West et al., 2000) as previously described (Blöthe et al., 2008). *Anaeromyxobacter*-specific PCR was performed according to the method described by Wu et al. (Ab112F, Ab227R; 2006).

2.7 Clone library construction

PCR amplicons produced with group-specific 16S rRNA gene primers were cloned using the pGEM®-T vector and *Escherichia coli* JM109 competent cells according to the manufacturer's instructions (Promega, Madison, WI USA). Clone libraries were screened by restriction fragment length analysis (RFLP) as previously described (Blöthe et al., 2008). All clones screened using RFLP were grouped into phylotypes according to RFLP banding patterns.

2.8 Phylogenetic and statistical analyses

Representative clones for each RFLP phylotype were sequenced bidirectionally using a Big-Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) on an Applied Biosystems 3100 Genetic Analyzer with Capillary Electrophoresis. Sequences were assembled using Sequencher v4.5 (Gene Codes Corp., Ann Arbor, MI) and prior to phylogenetic analysis, vector sequences flanking the 16S rRNA gene inserts were removed. Previously identified sequences with high sequence similarity to the clones obtained in this study were determined using the BLAST algorithm against the GenBank database available from National Center for Biotechnology Information (NCBI) (Altschul et al., 1990). Clone sequences were checked for chimeras and aligned with reference sequences in the ARB software package as previously described (Blöthe et al., 2008). Dendrograms were constructed in the ARB software package by adding 16S rRNA sequences to the distance-matrix tree using PARSIMONY_INTERAKTIV without changing the overall tree topology (Ludwig et al., 2004). The coverage of the clone libraries were calculated (Singleton et al., 2001), and the sampling efficiency within clone libraries was assessed using Analytica Rarefaction 1.3 software (http://www.uga.edu/strata/software/) originally derived by Heck et al. (1975).

2.9 Nucleotide sequence accession numbers

The 16S rRNA gene sequences presented in this study have been deposited in the EMBL database under the accession numbers AM941453-AM941457 for *Acidiphilium*-affiliated 16S rRNA gene sequences, AM941458-AM941489 for *Geobacter*-affiliated 16S rRNA gene sequences, and AM941490-AM941491 for *Geothrix*-affiliated 16S rRNA gene sequences.

3 Results

3.1 Fe(III)-reducing activities

The intermittent seep and the lowland fen displayed high Fe(II) formation rates during the first 7 days of incubation and reached high amounts of Fe(II) formed compared to the upland fen (Table 2). The lowland fen was depleted in nitrate, and consumption of sulfate started after Fe(II) formation reached the plateau (Fig. 1). However, CH_4 formation paralleled Fe(III) reduction in all soil depths (Fig. 1, and data not shown). These parallel activities were not observed in the intermittent seep and the upland fen. The upland fen displayed higher sulfate-consuming activities than the lowland fen (Table 2). The intermittent seep showed negligible in situ methane forming activities.

The initial rate of Fe(II) formation in the topsoil of the lowland fen was even slightly higher in March 2002 [1840 nmol g (wet wt soil)$^{-1}$ d^{-1}] than in September 2001 (Table 2). Apparently, the topsoil was more oxidized in March, because the initial Fe(II) concentration approximated only 1.7 μmol g (wet wt soil)$^{-1}$ compared to 7.3 μmol g (wet wt soil)$^{-1}$ in September. However, both experiments yielded similar maximum concentrations of Fe(II) at the end of incubation (Figs. 1 and 2) which were equivalent to 70% of the Fe_d content of the soil.

3.2 Source of iron in the lowland fen

To detect the source of the high Fe_d concentrations in the lowland fen (Table 1), 3 intermittent seeps were sampled upstream of the lowland fen, and the upper 5 to 15 cm layer of each soil was incubated under anoxic conditions. These soils formed Fe(II) without delay with rates ranging from 90 to 1050 nmol g (wet wt soil)$^{-1}$ d^{-1}. Two of these soils had high initial concentrations of Fe(II) indicating that Fe(III) reduction was an ongoing process in these wetland soils. The amounts of pedogenic iron (Fe_d) and oxalate extractable iron (Fe_o in these acidic (pH 3.2) soils ranged from 2.3 to 13.9 g kg^{-1} and from 1.8 to 12.5 g kg^{-1}, respectively.

3.3 Porewater biogeochemistry

Porewater depth profiles at the intermittent seep showed typical biogeochemical gradients of redox sensitive compounds indicating the sequential utilization of nitrate, Fe(III), and sometimes of sulfate with increasing soil depth during water saturated conditions between autumn (November) and early summer (June) (data not shown; Küsel and Alewell, 2004). Fe(II) reached maximum concentrations of 50 μM in 25 cm depth. Declining sulfate gradients were only detected in early summer samplings. Formate (up to 650 μM), acetate (up to 260 μM), and lactate (up to 85 μM), but not propionate or butyrate were detected in the upper 20 cm of the porewater.

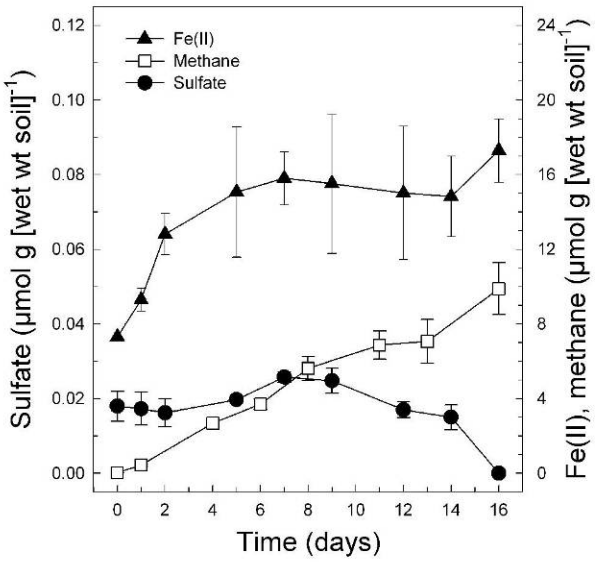

Fig. 1. Formation of Fe(II) and CH_4 and consumption of sulfate in anoxic microcosms of soil obtained from the lowland fen (0–10 cm depth) in September 2001. Presented are the averages ± standard deviations of triplicates.

In general, the upland and the lowland fen showed stronger reduced conditions than the seep. Both fens showed similar qualitative biogeochemical porewater patterns during the three year sampling period. However, the lowland fen showed up to 35-fold higher concentrations of Fe(II), up to 3-fold lower concentrations of sulfate, and up to 2-fold higher concentrations of CH_4 in the porewater than the upland fen. The concentrations of Fe(II) in the lowland fen were highly variable (Figs. 3 and 4). During summer of 2001, 2002, and 2004, the depth integrated (0-to-40 cm) average Fe(II) concentrations approximated 2961, 216, and 92 μM. In general, maximum Fe(II) concentrations occurred below 30 cm depth.

In the lowland fen, the concentrations of nitrate (up to 55 μM), sulfate (up to 140 μM), and negligible concentrations of Fe(II) and ammonia indicated soil oxygenation down to a depth of 25 cm after the snowmelt in 2002, 2003, and 2004 (Fig. 3). Oxygenation might have occurred due to mixing of the porewater with lateral flowing, oxygenated surface water. Drying of the topsoil (upper 5-to-10 cm) occurred during the hot summer in 2003 followed by heavy rain falls prior to sampling in September. Up to 420 μM sulfate (Figs. 3 and S1, http://www.biogeosciences.net/5/1537/2008/bg-5-1537-2008-supplement.pdf) were detected in the upper 20 cm of the fen soil, and Fe(II) concentrations did not exceed 30 μM. Fe(II) concentrations increased again in December 2003 to 370 μM. The concentration of sulfide never exceeded the detection limit of 5 μM in the porewater.

The concentrations of short chain fatty acids detected in the porewater of the lowland fen were much higher than in

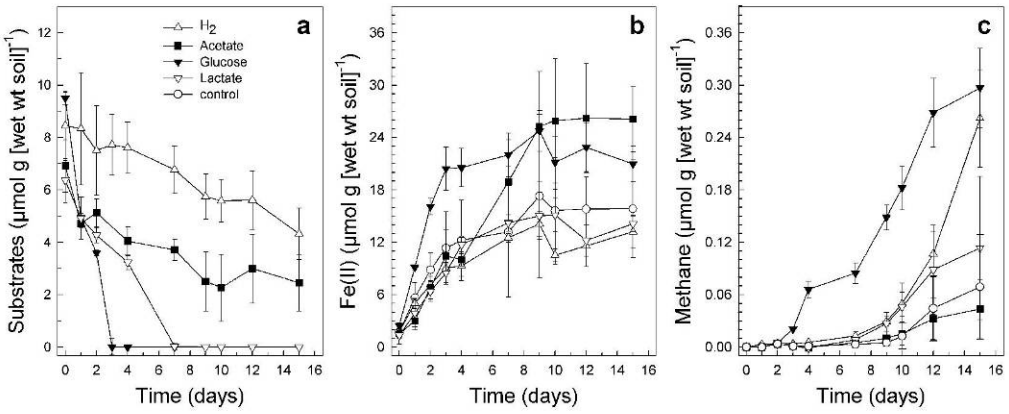

Fig. 2. Effect of the consumption of supplemental electron donors (**a**) on the formation of Fe(II) (**b**) and formation of CH_4 (**c**) in anoxic microcosms of soil obtained from the lowland fen (0–10 cm) in March, 2002. Presented are the averages ± standard deviations of triplicates.

the upland fen. In general, concentrations increased with increasing soil depth (Fig. 3). Depth integrated average concentrations in the lowland fen approximated 82, 56, 44, 15, and 4 μM during 2001 to 2004 for acetate, formate, lactate, propionate, and butyrate, respectively. Highest concentrations were detected in November 2001 and December 2002, where concentrations of acetate, propionate, butyrate, and lactate reached up to 3160, 1600, 100, and 95 μM in the porewater (Figs. 3 and 4b, and data not shown). In contrast, maximum concentrations of formate occurred in April 2002 (Fig. 3). Ethanol was never detected. Porewater pH did not decrease in the presence of high concentrations of short chain fatty acids. Acetate concentrations were positively correlated with Fe(II) (R^2=0.75; Fig. 4).

3.4 Effect of supplemental electron donors on Fe(III)-reducing activities

Since the high concentrations of acetate observed might be derived from fermentation, glucose and typical fermentative products were supplemented to lowland fen soil. The initial rate of Fe(II) formation was enhanced from 1.84 μmol g (wet wt soil)$^{-1}$ d^{-1} to 6.08 μmol g (wet wt soil)$^{-1}$ d^{-1} by supplemental glucose, but not with supplemental acetate, lactate, and H_2. However, a secondary Fe(II)-forming increase occurred after 4 days in acetate microcosms (Fig. 2). Glucose and acetate supplemented microcosms yielded higher maximum Fe(II) concentrations of 24.7 and 26.2 μmol g (wet wt soil)$^{-1}$ compared to 15.4 μmol g (wet wt soil)$^{-1}$ of the control.

Glucose was rapidly consumed within 3 days (Fig. 2a) and yielded CO_2 and acetate as end products, and H_2 and ethanol as transient products (Fig. S2, http://www.biogeosciences.net/5/1537/2008/bg-5-1537-2008-supplement.pdf). Supplemental lactate yielded acetate as main product. Both acetate and H_2 were not completely consumed during 15 days of incubation. H_2 but not acetate stimulated the formation of CH_4 (Fig. 2c). Microcosms supplemented with either acetate, H_2 or glucose with soil obtained in October 2002 yielded similar results (data not shown). Formate was detected as a small transient product only in glucose amended microcosms of October 2002.

3.5 Molecular detection of Fe(III) reducers

PCR with lowland fen soil yielded products with 16S rRNA primers specific for acidophiles belonging to *Acidiphilium* and for neutrophiles belonging to *Geobacter* or *Geothrix*. No PCR products were obtained with a primer set specific for bioleaching-associated bacteria, *Shewanella* or *Anaeromyxobacter*. PCR products with *Geobacter* specific primers were detected in enrichments obtained from a 10^{-3} soil dilution transferred to mineral medium at pH 5.5 supplemented with 40 mM amorphous ferric hydroxide [$Fe(OH)_3$] and 5 mM acetate or H_2. PCR products with *Acidiphilium* specific primers were detected up to a 10^{-3} soil dilution enrichment amended with acetate but not with H_2, and PCR products with *Geothrix* specific primers were detected only in 10^{-1} soil dilution enrichments. Screening of 16S rRNA gene clones by RFLP revealed that all phylotypes detected in the enrichments also occurred in the fen soil.

A total of 45 *Acidiphilium*-16S rRNA gene clones were screened by RFLP, and 13 different phylotypes could be differentiated. Comparative sequence analyses indicated that 5 phylotypes were 95% similar to cultured *Acidiphilium* or *Acidosphaera* species. Clones were most closely related (96–98% sequence similarity) to a forest soil or sphagnum peat bog clone (Fig. 5). With the primer pair specific for *Geothrix*, 20 clones were obtained, but only 2 different phylotypes were obtained with a 96–97% sequence similarity to *Geothrix*-related sequences.

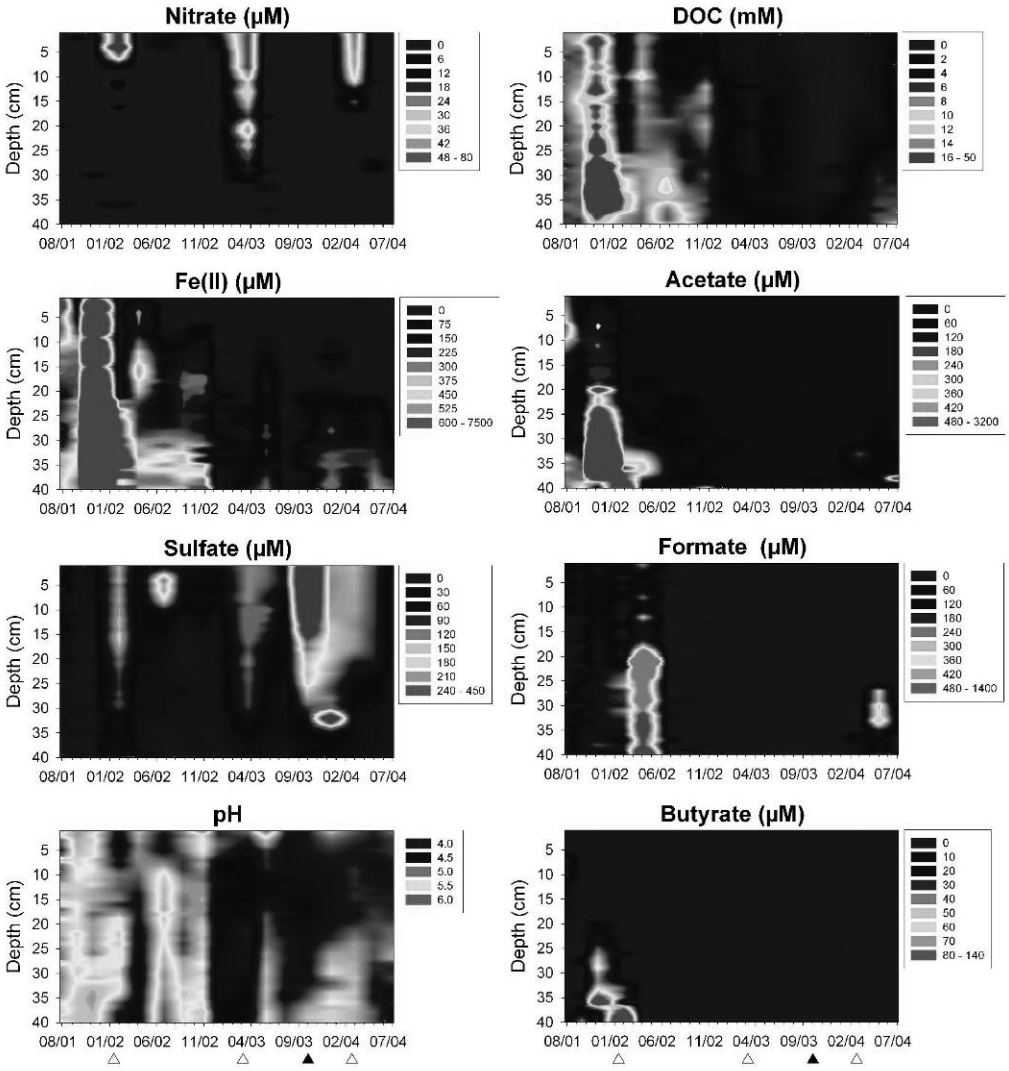

Fig. 3. Porewater concentrations of nitrate, Fe(II), sulfate, pH, DOC, acetate, formate, and buturate in the lowland fen sampled during end of July 2001 to July 2004. Please note that the upper concentrations were combined in a range for better visualization of low concentrations. White triangles below the x-axis flag the snow melt events, the black triangle flags the summer drought followed by a heavy rain fall in September 2003.

With the primer pair specific for *Geobacter*, a total of 83 clones were obtained and 40 different phylotypes were obtained. A number of non-*Geobacteraceae* sequences and chimeras between *Geobacteraceae* and non-*Geobacteraceae* were detected. Comparative sequence analysis revealed that 34 of the 40 sequences retrieved showed high sequence identity to *Geobacteraceae* sequences (Fig. 6); one was related to *Geobacter chapellii* str. 172 (96% sequence similarity), three were related to *Geobacter bemidjiensis* and *Geobacter bremensis* (94–96% sequence similarity). Many sequences were similar to *Pelobacter* spp. (94–97%). *Acidiphilium* and *Geobacter* specific 16S rRNA gene clone libraries showed coverages of 57 and 35%, respectively.

4 Discussion

4.1 Mobilization and oxidation of Fe(II) in the catchment

At the upland fen, porewater concentrations of Fe(II) were low, similar to oligotrophic ombrogenic peatlands that receive most of their iron from atmospheric deposition. The low Fe(III) reduction rates corresponded to the low amounts of oxalate extractable Fe(III) oxides present in the upland fen and indicated that Fe(III) reduction was of minor significance for the oxidation of carbon in this fen similar to other northern peatlands (Blodau et al., 2002). Porewater profiles of Fe(II) and sulfate in the intermittent seep were similar to pH

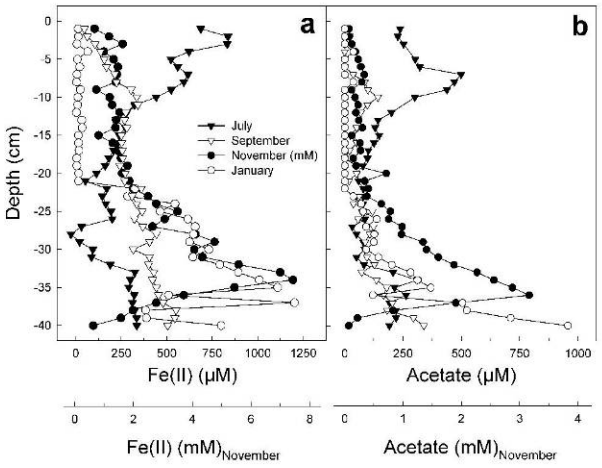

Fig. 4. Detailed porewater depth profiles of Fe(II) **(a)** and acetate **(b)** in the lowland fen sampled in July, September, and November 2001, and in January 2002.

neutral fens (Todorova et al., 2005; Dettling et al., 2006) or other wetlands (Roden and Wetzel, 2003). The high Fe(II) formation rate at the seep suggested a mobilization of Fe(II) during waterlogging from autumn to spring. Fe(II) was also spontaneously formed in other waterlogged seeps sampled upstream of the lowland fen further strengthening the suggestion that Fe(III) reduction is an ongoing process in many seeps of this catchment. The lowland fen is connected to a shallow groundwater layer and receives water from these intermittent seeps and fens located in the north-east of the Lehstenbach catchment (Küsel and Alewell, 2004). Due to the acidic (pH 3.1) soil conditions of most seeps, the majority of the reductive dissolved Fe(II) will not adsorb to the solid phase and move with the groundwater flow. Indeed, concentrations of Fe(II) in a nearby groundwater well range from 9-to-143 μM (Küsel and Alewell, 2004). Thus, the lowland fen appears to receive continuously anoxic Fe(II)-rich groundwater. The high accumulation of iron in the upper soil of the lowland fen might have resulted from the oxidation of Fe(II) in oxidized peat surface layers. The high Fe_o/Fe_d ratios (~0.8) (Table 1) suggest that Fe(III) precipitated as amorphous oxides or as organic matter complexes. Microbial Fe(II) oxidation might yield colloidal or dissolved forms of Fe(III) readily available for microbial reduction (Roden et al., 2004). The high mean DOC concentration (77 mg L^{-1}) in the porewater would further favor Fe(III) reduction, because humic compounds can serve as electron shuttles between Fe(III) reducers and surface-bound Fe(III) sterically not accesible to microorganisms (Lovley et al., 2004).

4.2 In situ relevance of Fe(III)-reducing activities

According to the 1:4 ratio of CO_2 production to Fe(III) reduction (Roden and Wetzel, 1996), microbial Fe(III) reduction in the most active upper soil layer (0–10 cm) accounted for 26.7, 6.7, and 71.6% of the anaerobic organic carbon mineralization in the intermittent seep, the upland, and the lowland fen, respectively. In rhizosphere and unvegetated wetland sediments Fe(III) reduction accounts for approximately 65 and 40% of carbon mineralization (Roden and Wetzel, 1996). Thus, Fe(III) reduction can substantially contribute to carbon mineralization also in peatlands and reduce CH_4 emissions. The lowland fen showed sequential Fe(III)-reducing and sulfate-reducing activities but concomitant Fe(III)-reducing and methanogenic activities (Fig. 1). Overlapping or concomitant Fe(III)-reducing and methanogenic activities were not observed in soil samples obtained after the snow melts indicating that prolonged anoxic or reduced conditions in the fen were necessary for the establishment of methanogenic activities. Short-term oxygenation of reduced surface soil during summer that leads to a rapid renewal of the Fe(III) pool might yield a partial shift of the electron flow from CO_2 to Fe(III) by methanogens and help to explain concomitant reduction processes in peatlands (Dettling et al., 2006; Metje and Frenzel, 2005; Paul et al., 2006) or in rice paddy soils after drainage (Krüger et al., 2001). The ability of some methanogens to interact with extracellular quinones, humic acids, and Fe(III) oxides has raised the possibility that methanogens contribute to Fe(III) and humic acid reduction (Bond and Lovley, 2002; van Bodegom et al., 2004). Concomitant activities can be also explained by the use of non-competetive substrates. Concomitant or reversed Fe(III) and sulfate reduction is reported from other fens (Todorova et al., 2005). It was suggested that concomitant reduction processes are due to the presence of more crystalline Fe(III) oxides like hematite or goethite in the soil (Postma and Jakobsen, 1996), which are reduced at lower redox potentials (Straub et al., 2001) than sulfate or CO_2 (Zehnder and Stumm, 1988). However, the high Fe_o/Fe_d ratios in the top soil hint to amorphous, easily reducible Fe(III) oxides.

4.3 Accumulation of acetate

High short chain fatty acid and high Fe(II) concentrations were detected in deeper soil of the lowland fen during autumn to winter in 2001/2002 and 2002/2003 with maximum concentration of 3 and 7 mM for acetate and Fe(II), respectively. The high Fe(II) concentrations in this depth were surprising due to the relatively low amounts of iron in the solid phase. However, porewater biogeochemical gradients in fens are not only based on diffusion like in lake sediments. We can not rule out that a part of the Fe(II) was transported with the groundwater flow. Acetate could have been produced by fermentors using plant polysaccarides from dead

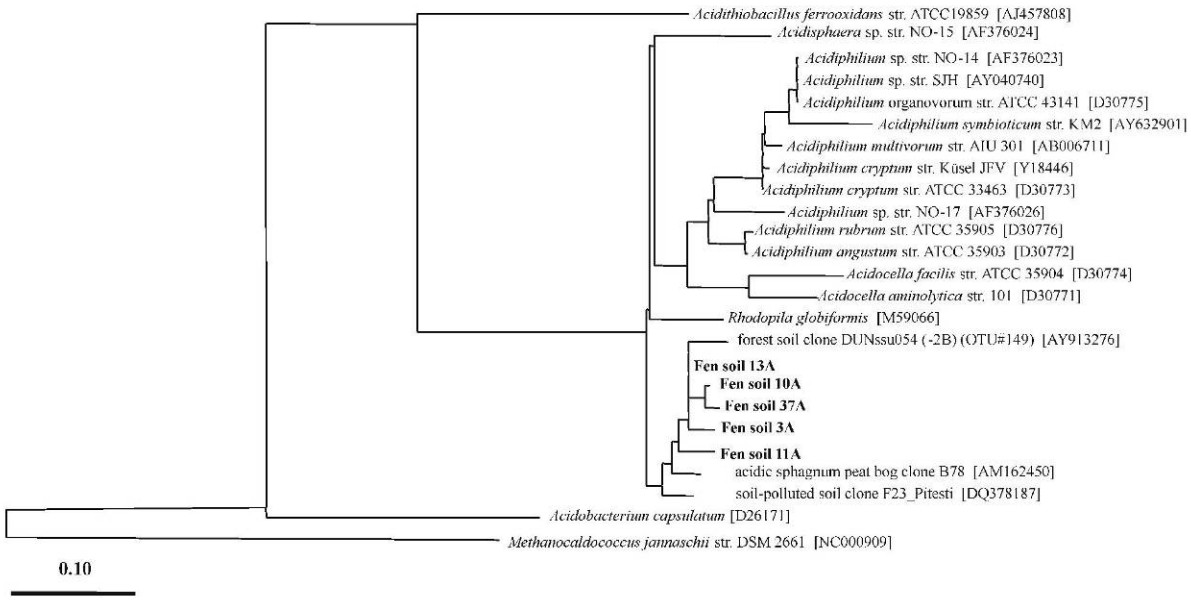

Fig. 5. Phylogenetic tree showing the relative positions of *Acidiphilium*-affiliated 16S rRNA gene sequences derived from the lowland fen soil (0–10 cm) obtained in October 2003. Sequences were added to the existing tree without changing the overall tree topology by using the ARB treeing tool PARSIMONY_INTERAKTIV. Names and accession numbers (between bracktes) for closest relatives 16S rRNA gene sequences are given. The bar indicates 10% sequence divergence.

root material. Accumulation of acetate up to 1.2 mM is reported from bogs in Michigan or Ontario (Shannon and White, 1996; Blodau et al., 2002) and appears to be due to the absence of acetoclastic methanogenesis (Shannon and White, 1996; Hines et al., 2001). Temporal acetate accumulations might characterize fens and bogs as habitats with specific qualities. The decoupling of methanogenesis from carbon flow might be due to the direct inhibition of accumulated acetate on methanogens, because acetic acid can penetrate through the cell membrane and act as decoupler of the proton motive force under acidic conditions (Williams and Crawford, 1984; Goodwin and Zeikus, 1987) or due to transport and thermodynamic constraints (Beer and Blodau, 2007).

The positive correlation of acetate and Fe(II) in the lowland fen porewater during the 3-year-period (Figs. 3 and 4) suggests either a formation of acetate by incomplete oxidizing Fe(III) reducers or an accumulation of acetate after depletion of the Fe(III) pool by complete acetate-oxidizing Fe(III) reducers. Cultured *Geobacter* species, which were most closely related to clone sequences retrieved from this fen, like *G. chapellii*, *G. bemidjiensis*, and *G. bremensis* are complete oxidizers (Lovley et al., 2004). In contrast, the also closely related *Pelobacter venetianus* is an incomplete oxidizer. The low or negligible concentrations of acetate in upper soil suggests oxidation of acetate to CO_2 via aerobic respiration, other oxidative microbial processes or syntrophic oxidation of acetate to CO_2 by the concerted activity of acetate-oxidizing anaerobes and hydrogenotrophic methanogens (Horn et al., 2003; Metje and Frenzel, 2007).

4.4 Fe(III)-reducing microbial communities of acidic habitats

Due to our limited knowledge about Fe(III) reduction in moderate acidic habitats, phylogenetic analyses of Fe(III) reducers based on known 16S rRNA gene sequences are severely limited, and we might miss important genera. Phylotypes related to cultured *Acidiphilium* or *Acidisphaera* species were detected in the lowland fen similar to slightly acidic coal mining lake sediments (Blöthe et al., 2008). Most Fe(III)-reducing prokaryotes cultured to date are either neutrophilic or acidophilic and have only minor capacities to reduce Fe(III) at in a pH range from 4 to 6. The acidophilic *Acidiphilium cryptum* (ATCC 33463) can reduce only small amounts of solid phase Fe(III) at pH 5 (Bilgin et al., 2004). *Geobacter* sp. CdA-3 that was isolated from a mining-impacted sediment can reduce Fe(III) at a pH range from 5.5 to 8.1 (Cummings et al., 2000), and *G. bremensis* can reduce Fe(III) down to pH 5 (Straub and Bucholz-Cleven, 2001). Members of the *δ-Proteobacteria* subdivision, including *Geobacter*- and *Anaeromyxobacter dehalogenans*-related sequences seem to be important metal-reducing organisms in biostimulated acidic subsurface sediments (North et al., 2004) However, other studies with acidic uranium contaminated sediments demonstrate that

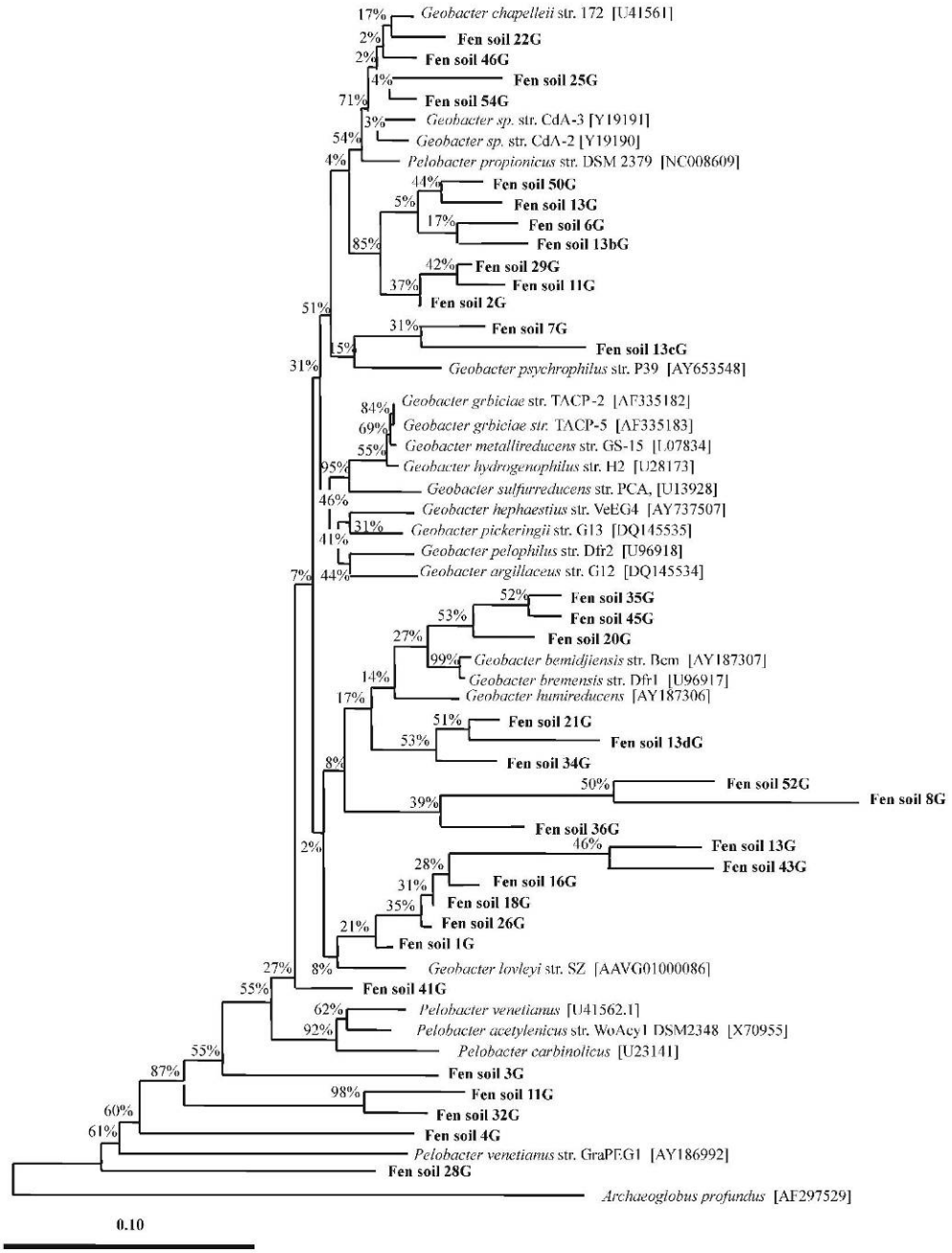

Fig. 6. Phylogenetic tree showing the relative positions of the *Geobacter*-affiliated 16S rRNA gene sequences derived from the lowland fen soil (0–10 cm) obtained in October 2003 as inferred by Parsimony method. Bootstrap values for a total of 100 replicates are shown at the nodes. Names and accession numbers (between bracktes) for all 16S rRNA gene sequences used for comparsion are given. The bar indicates 10% sequence divergence.

Geobacteraceae dominate only in sediment enrichment cultures incubated under neutral pH conditions (Petrie et al., 2003). Surprisingly, no PCR products of *Anaeromyxobacter*, or *Shewanella* related species were obtained from the lowland fen, although microorganisms from these genera are common to various metal-reducing environments. Recently it was shown that *Acidobacterium capsulatum* is capable of Fe(III) reduction in a pH range of 2 to 5 (Blöthe et al., 2008)

and that the potential for dissimilatory Fe(III) reduction is widespread among acidophilic heterotrophic bacteria (Coupland and Johnson, 2008). *Acidobacteria* are present in peatlands (Dedysh et al., 2006) and have been detected also in the upland fen of this catchment (Schmalenberger et al., 2008). Thus, results from phylogenetic analyses in this investigation provide an incomplete picture.

5 Conclusions

Our field based experimental results corroborate the hypotheses that a decrease in annual summer precipitation in most northern European regions as a result of global climate change (IPCC 2007) will shift the electron flow away from methanogenesis to alternative anaerobic processes in peatlands. Drying of the top soil down to 10 cm depth during the hot summer 2003 followed by heavy rain falls in September lead to the appearance of sulfate in the porewater and a renewal of Fe(III) which favored the subsequent Fe(III)- and sulfate-reducing activities. Drying and rewetting of the top soil 2003 yielded even higher porewater sulfate concentrations in the lowland fen than in the upland fen despite the average 3-fold lower sulfate concentrations in the lowland fen (Fig. S1). The higher release of sulfate might be due to differences in the reduced sulfur pools, which were subjected to oxidation. The Fe-rich lowland fen contains higher contents of acid volatile sulfur (AVS, i.e. amorphous FeS) and total reduced inorganic sulfur (TRIS, i.e. amorphous FeS, S^0, and FeS_2) (Loy et al., 2004) but lower contents of organic sulfur compared to the upland fen (Paul et al., 2006). Synchrotron-based X-ray spectromicroscopy revealed that the fraction of organic reduced sulfur of these fens is more stable under alternating reduction-oxidation processes than the FeS or FeS_2 pool (Prietzel, personal communication), similar to results obtained with chemical S fractionations in peat bogs (Wieder and Lang, 1988; Wieder et al., 1990). Thus, the presence of Fe(II) seemed to affect both the storage and mobilization of sulphur. An increase of extreme weather conditions like summer droughts and heavy rainfall events during the next decades will amplify the importance of iron for biogeochemical processes in iron-rich peatlands.

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Supplementary Material

Microbial reduction of iron and porewater biogeochemistry in acidic peatlands

K. Küsel, M. Blöthe, D. Schulz, M. Reiche, and H. L. Drake

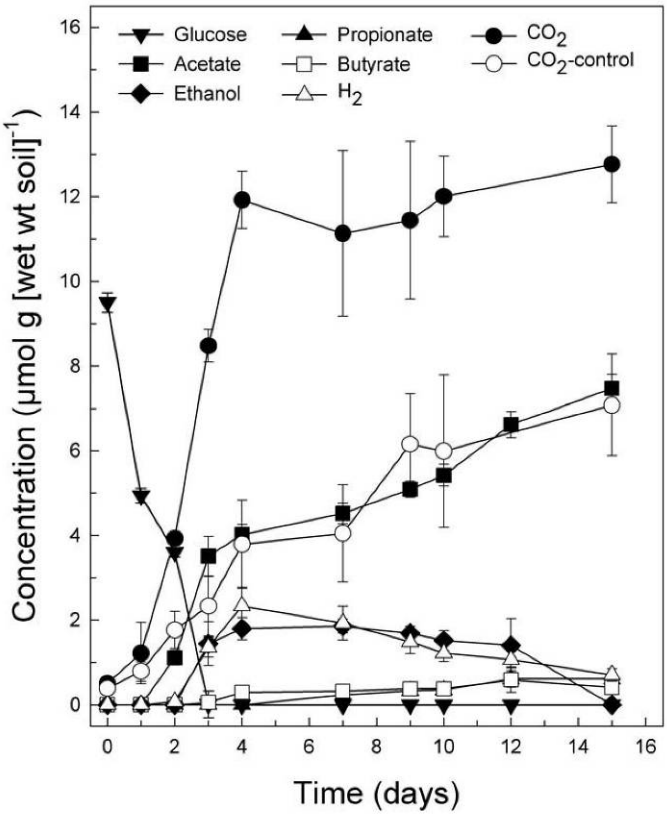

Fig. S1. Effect of the consumption of supplemental glucose on the formation of fermentation products in anoxic microcosms of soil obtained from the lowland fen (0-10 cm) in March 2002. Presented are the averages ± standard deviations of triplicates.

Supplementary Material

Microbial reduction of iron and porewater biogeochemistry in acidic peatlands

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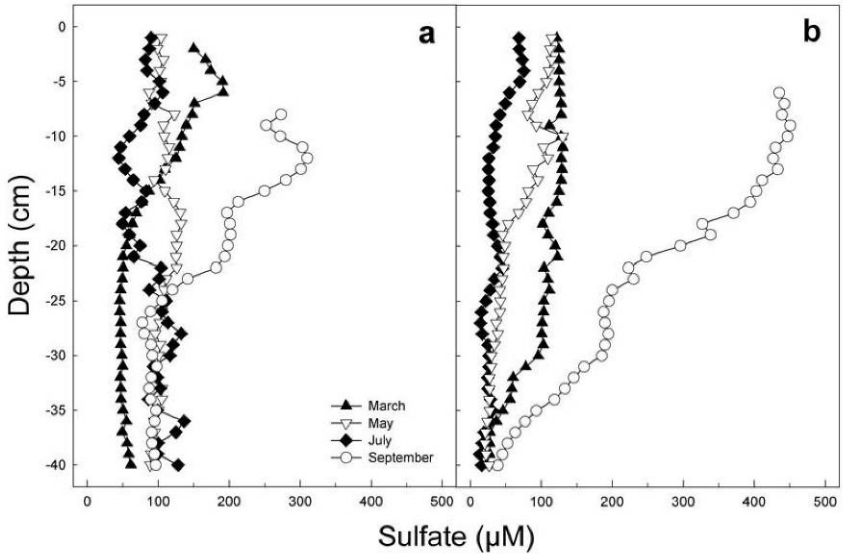

Fig. S2. Porewater depth profiles of sulfate in the upland (a) and lowland (b) fen sampled in March, May, July, and September 2003.

EIGENSTÄNDIGKEITSERKLÄRUNG

Ich versichere an Eides statt, dass ich die von mir vorgelegte Dissertation selbständig angefertigt und nur die von mir angegebenen Quellen und Hilfsmittel verwendet habe. Die Bestimmungen der Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena sind mir bekannt. Die Hilfe eines Promotionsberaters wurde nicht in Anspruch genommen und Dritte erhielten weder unmittelbar noch mittelbar geldwerte Leistungen, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen. Die Dissertation oder Teile davon wurde noch nicht als Prüfungsarbeit an der Friedrich-Schiller-Universität Jena oder an einer anderen Einrichtung für eine staatliche oder andere wissenschaftliche Begutachtung eingereicht.

Jena, den 08.01.2009

Ort, Datum

Marco Reiche

HERVORGEGANGENE UND GEPLANTE PUBLIKATIONEN

Alle Kapitel der Dissertation sind bzw. werden als Publikationen bei internationalen Fachzeitschriften eingereicht. Mein Beitrag an der Erstellung der vorliegenden sechs Manuskripte und Publikationen gestaltete sich wie folgt:

Küsel, K., Blöthe, M., Schulz, D., **Reiche, M.**, Drake, H.L. *Microbial reduction of iron and porewater biogeochemistry in acidic peatlands*. **Biogeosciences** 5:1537-1549 (2008):
Ermittlung der An- und Abwesenheit bekannter Gattungen von Fe(III)-reduzierenden Mikroorganismen mittels 16S rRNA PCR, Erstellung und Bearbeitung von einigen Grafiken sowie Korrektur des Manuskripts; der wesentliche Anteil der Durchführung und Planung sowie die Überarbeitung des Manuskriptes wurde von den Koautoren erarbeitet und diente als Grundlage für die Beantragung des DFG Projektes; Erstellung des Manuskriptes durch K. Küsel

Reiche, M., Torborg, G., Küsel, K. *Competition of Fe(III) reduction and methanogenesis in an acidic fen*. **FEMS Microbiology Ecology** 65:88-101 (2008):
Erstellung des Manuskripts, Durchführung und Planung des Hauptteiles der Freiland- und Laborarbeiten sowie Auswertung der Daten; substratinduzierte Methanbildung durch G. Torburg, konzeptionelle Abstimmung und Planung der Arbeiten sowie Überarbeitung des Manuskriptes durch K. Küsel

Reiche, M., Hädrich, A., Lischeid, G., Küsel, K. *Impact of manipulated drought and heavy rainfall events on peat mineralization processes and source-sink functions of an acidic fen*.
Journal of Geophysical Research – Biogeosciences (accepted in December 2008):
Erstellung des Manuskripts, Durchführung und Planung des Hauptteiles der Freiland- und Laborarbeiten sowie Auswertung der Daten; exoenzymatische Aktivitäten und Respirationsraten sind in Teilen durch A. Hädrich bestimmt worden; Wasserspiegeldaten, Wetterdaten und Instrumentierung der Versuchsflächen durch A. Liescheid; konzeptionelle Abstimmung und Planung der Arbeiten sowie Überarbeitung des Manuskriptes durch K. Küsel

Reiche, M., Gleixner, G., Küsel, K. *Link of peat quality to microbial respiration and methanogenesis in an acidic fen*. **Oecologia** (to be submitted):
Erstellung des Manuskripts, Durchführung und Planung der Freiland- und Laborarbeiten sowie Auswertung der Daten; Thermogravimetrie- und Pyrolyse/MS-Analysen wurden am Max-Planck Institut für Biogeochemie in der Arbeitsgruppe „Biogeochemische Prozesse“ durch G. Gleixner geleitet und Daten diskutiert; konzeptionelle Abstimmung und Planung der Arbeiten sowie Überarbeitung des Manuskriptes durch K. Küsel

Knorr, K.H., Liescheid, G., **Reiche, M.**, Küsel, K., Blodau, C. *Dynamics of belowground redox processes in a minerotrophic fen exposed to a water table manipulation*.
Biogeosciences (to be submitted):
Bereitstellung von Daten über Sauerstoffpenetrationstiefen während des Freilandexperimentes und Korrektur des Manuskriptes; Erstellung des Manuskripts und Durchführung des Hauptteiles der Freiland sowie Laborarbeiten durch K. H. Knorr; Korrektur des Manuskriptes durch K. Küsel; konzeptionelle Abstimmung und Planung der Arbeiten sowie Überarbeitung des Manuskriptes durch C. Blodau

Lüdecke, C. **Reiche, M.**, Torborg, G., Küsel, K. A*ctivity and diversity of Fe(II)- oxidizers at oxic-anoxic gradienst in an acidic fen*.
Applied and Environmental Microbiology (in preparation):
Erstellung des Manuskripts, Planung der Laborarbeiten sowie Einweisung in die Methoden; Durchführung der Laborarbeiten sowie Auswertung der Daten durch C. Lüdecke; Etablierung von Methoden und Anreicherung von Kulturen durch G. Torborg; konzeptionelle Abstimmung und Planung der Arbeiten sowie Überarbeitung des Manuskriptes durch K. Küsel

Bestätigung des Eigenanteils an den Manuskripten:

Jena, den 08.01.2009

Ort, Datum	Kirsten Küsel

DANKSAGUNG

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CURRICULUM VITAE

Persönliche Daten

Marco Reiche
geboren am 04.05.1979 in Berlin

Bildungsweg

seit 2005 Doktorand an der Friedrich-Schiller-Universität Jena, Biologisch-Pharmazeutische Fakultät, Institut für Ökologie, AG Limnologie

Thema: *Microbial mineralization processes influenced by water table changes and peat quality in an acidic fen*

Diese Arbeit wurde im Rahmen der Forschergruppe 562 *Dynamik von Bodenprozessen bei extremen meteorologischen Randbedingungen* angefertigt und durch die Deutsche Forschungsgemeinschaft (DFG) gefördert.

2004 - 2005 Masterstudium Fishery Science and Aquaculture an der Humboldt-Universität zu Berlin

Thema: Development of methods to determine microbial activities in peat

Abschluss: Master of Science

1999 - 2004 Studium der Fischwirtschaft und Gewässerbewirtschaftung an der Humboldt-Universität zu Berlin

Thema: Modifikation und Etablierung von Methoden zur Erfassung von mikrobiellen Aktivitäten in Torfen

Abschluss: Diplom Agraringenieur

1998 - 1999 Wehrdienst im Panzergrenadierbataillon 182, Bad Segeberg

1991 - 1998 Max-Reinhardt-Oberschule in Berlin - Hellersdorf,
Abschluss: Allgemeine Hochschulreife (Abitur)

Studienbegleitende Tätigkeiten

Fortbildungen

03/2008 SCIENCE^{PLUS}-Workshop, *Team- & Führungskompetenz in der Hochschule und außerhalb*, Friedrich-Schiller-Universität Jena

06/2007 Forschungsaufenthalt an der School of Biological Sciences, University of Wales, Prof. C. Freeman, *Measurement of Phenoloxidases*, Bangor (GB)

03/2007 Workshop, *9. Tag der Mikroskopie*, Carl-Zeiss, Jena

Lehre

Graduierungsarbeiten

2008 *Activity and diversity of Fe(II) oxidizers at oxic-anoxic interfaces obtained from an acidic fen*, Betreuung von Claudia Lüdecke im Rahmen einer Diplomarbeit

2007 *Exoenzymatic activities and carbon dioxide formation during a drying and rewetting experiment in a slightly acidic fen*, Betreuung von Oliver Feig im Rahmen einer Bachelorarbeit

Aspects of iron cycle and methanogenesis in an acidic fen, Betreuung von Grit Torburg im Rahmen einer Diplomarbeit

2006 *Microbial mineralization processes in iron-rich fens subjected to water table manipulations*, Betreuung von Anke Hädrich im Rahmen einer Diplomarbeit

Praktika

2007 *Mikrobielle Aktivitäten in einem Niedermoor bei einem Austrocknungs- und Wiedervernässungsexperimentes*, Betreuung von Jennifer Schmidt und Kerstin Pasemann im Rahmen des GP II, gesamtes Sommersemester

300 µm surface-layer oxydized sediment gained greateffects in P-retention at a eutrophic shallow lake, Betreuung von Christian Kaufmann und Tina Keller im Rahmen des GP III, gesamtes Sommersemester

Einfluss von Huminstoffen auf exoenzymatische Aktivitäten, Betreuung von Oliver Feig im Rahmen eines Laborpraktikums, 2 Monate

Charakterisierung eines Fließ- und Stillgewässers, Betreuung von 10 Studenten im Rahmen des Geländepraktikums, 3 Tage

Stratifikation von Seen - Aquarienversuch, Betreuung der 12 Teilnehmer im Rahmen des GP I, 1 Tag

2006 *Baden oder nicht baden?* - Nährstoffdynamik im Porstendorfer See, Betreuung von 6 Praktikanten im Rahmen des GP II, gesamtes Sommersemester

Betreuung von Grit Neumann und Katrin Dix im Rahmen das Alternativpraktikums für Limnologie, 10 Tage

Betreuung von Melanie Wirth im Rahmen eines Laborpraktikums, 3 Monate

Substratinduzierte Fe(III)-Reduktion, Betreuung von Sylvia Löffler im Rahmen des GP III, 1 Monat

Vorlesungen

2008 *Aktuelle Entwicklungen in der angewandten Limnologie*, Betreuung von 30 Studierenden im Rahmen eines Seminars, 2 SWS, gesamtes Sommersemester

01/2007 *Struktur und Funktion von Fe(III)-Reduzierern und Methanogenen in einem Niedermoor*, 2 h im Rahmen der Doktoranden-Ringvorlesung

12/2005 *Struktur und Funktion von Fermentierern, Fe(III)-Reduzierern und Methanogenen in einem Niedermoor*, 2 h im Rahmen der Doktoranden-Ringvorlesung

Sonstiges

2005 - 2008 Administration und Gestaltung der Arbeitsgruppenhomepage

Beisitzer in Diplomprüfungen im Hauptfach und Nebenfach Ökologie

Betreuung von studentischen Hilfskräften

Tagungen und Präsentationen

Vorträge

2008 M. Reiche, C. Lüdecke and K. Küsel: *Microbial Iron Cycling at the Oxic-Anoxic Interface in an Acidic Fen*, 12th International Symposium on Microbial Ecology – ISME 12, Cairns, Australia, August 17-22

2007 M. Reiche and K. Küsel: Institut für Ökologie *Artificial climate change: What a defenceless fen has to tolerate*, Annual Symposium of the Institute of Ecology, Jena, Germany, December 17-18

C. Blodau, G. Lischeid, K.H. Knorr, M. Reiche, K. Küsel, S. Goldberg, J. Muhr, K. Hentschel, W. Borken, G. Gebauer, C. Weyer, A. Hamberger, M. Horn, H.L. Drake, S. Peiffer, E. Matzner: *Impact of experimental drainage and rewetting on biogeochemical processes in a northern fen,* International Symposium on Soil Processes under Extreme Meteorological Conditions, Bayreuth, Germany, February 25-28

2006 M. Reiche und K. Küsel: *Methanbildung und Reduktion von Eisen(III) in einem schwach sauren Niedermoor*, Workshop für Stoffumsatzprozesse in Mooren und ihr Einfluss auf angrenzende Gewässer, Berlin, Deutschland, 19.-20. April

2005 - 2007 M. Reiche und K. Küsel: Kurzvorträge bei offiziellen Treffen der Forschergruppe 562 „Bodenprozesse bei extremen meteorologischen Randbedingungen der Deutschen Forschungsgemein-schaft (DFG)“ im Juni und November 2005, im Juli und Dezember 2006 und im Februar, Juli und November 2007

Poster

2008 C. Lüdecke, M. Reiche and K. Küsel: *Activity and Diversity of Ironoxidizers obtained from Oxic-Anoxic Interfaces of an Acidic Fen*, Deutsche Mineralogische Gesellschaft (DMG), Berlin, Germany, September 15-17

2007 M. Reiche and K. Küsel: *Stimulation of Microbial Fe(III)-Reduction in an Acidic Fen after a Drying and Rewetting Cycle*, International Symposium on Soil Processes under Extreme Meteorological Conditions, Bayreuth, Germany, February 25-28

M. Reiche and K. Küsel: *Sampling Design for Microbial and Geochemical Parameters in an Acidic Fen during a Drying and Rewetting Cycle,* International Symposium on Soil Processes under Extreme Meteorological Conditions, Bayreuth, Germany, February 25-28

A. Sieler, M. Reiche and K. Küsel: *Exoenzymatic Activities during a Drought and Rewetting Experiment in a Slightly Acidic Fen,* International Symposium on Soil Processes under Extreme Meteorological Conditions, Bayreuth, Germany, February 25-28

2006 M. Reiche and K. Küsel: Institut für Ökologie *Artificial climate change: Microbial Reduction of Iron(III) in a Methane-emitting Acidic Fen* (updated), Annual Symposium of the Institute of Ecology, Jena, Germany, December 18-19

M. Reiche and K. Küsel: *Microbial Reduction of Iron(III) in a Methane-emitting Acidic Fen* (updated), Deutsche Gesellschaft für Limnologie - DGL, Dresden, Germany, September 25-29

M. Reiche and K. Küsel: *Microbial Reduction of Iron(III) in a Methane-emitting Acidic Fen*, Annual Conference of the Association for General and Applied Microbiology - VAAM, Jena, Germany, March 19-22

Jena, den 08.01.2009

Ort, Datum

Marco Reiche